REVIEW ARTICLE

Roles, and establishment, maintenance and erasing of the epigenetic cytosine methylation marks in plants

SUSHIL KUMAR[1,2]*, RENU KUMARI[1,3], VISHAKHA SHARMA[1,2] and VINAY SHARMA[3]

[1]*National Institute of Plant Genome Research (NIPGR), Aruna Asaf Ali Marg, New Delhi 110 067, India*
[2]*SKA Institution for Research, Education and Development (SKAIRED), 4/11 Sarv Priya Vihar, New Delhi 110 016, India*
[3]*Banasthali University, P. O. Banasthali Vidhyapeeth, Banasthali 304 022, India*

Abstract

Heritable information in plants consists of genomic information in DNA sequence and epigenetic information superimposed on DNA sequence. The latter is in the form of cytosine methylation at CG, CHG and CHH elements (where H = A, T or C) and a variety of histone modifications in nucleosomes. The epialleles arising from cytosine methylation marks on the nuclear genomic loci have better heritability than the epiallelic variation due to chromatin marks. Phenotypic variation is increased manifold by epiallele comprised methylomes. Plants (angiosperms) have highly conserved genetic mechanisms to establish, maintain or erase cytosine methylation from epialleles. The methylation marks in plants fluctuate according to the cell/tissue/organ in the vegetative and reproductive phases of plant life cycle. They also change according to environment. Epialleles arise by gain or loss of cytosine methylation marks on genes. The changes occur due to the imperfection of the processes that establish and maintain the marks and on account of spontaneous and stress imposed removal of marks. Cytosine methylation pattern acquired in response to abiotic or biotic stress is often inherited over one to several subsequent generations. Cytosine methylation marks affect physiological functions of plants via their effect(s) on gene expression levels. They also repress transposable elements that are abundantly present in plant genomes. The density of their distribution along chromosome lengths affects meiotic recombination rate, while their removal increases mutation rate. Transposon activation due to loss of methylation causes rearrangements such that new gene regulatory networks arise and genes for microRNAs may originate. Cytosine methylation dynamics contribute to evolutionary changes. This review presents and discusses the available evidence on origin, removal and roles of cytosine methylation and on related processes, such as RNA directed DNA methylation, imprinting, paramutation and transgenerational memory in plants.

[Kumar S., Kumari R., Sharma V. and Sharma V. 2013 Roles, and establishment, maintenance and erasing of the epigenetic cytosine methylation marks in plants. *J. Genet.* **92**, 629-666]

Introduction

Phenotypic variation in a plant genotype, arising from differential pattern of cytosine methylation on DNA and/or differences in patterning of histone modifications in chromatin is referred to as epigenetic variation. The variation in epigenetic patterns results from changes in the environments or developmental transitions or may arise spontaneously. Depending on its nature, an epigenetic feature may be inherited transiently through a small number of mitotic divisions or inherited stably over various developmental phases and transferred through gametes to immediate progeny or persist over several generations until the distinctive feature is changed (figure 1; table 1). The variation in DNA methylation patterns arises because not all of the putative cytosine sites get methylated and maintenance of methylation is error prone. Stability of methylation patterns at loci is determined by the site-specific DNA sequence. Methylation marks at some loci may be very stable. Variation in the histone modification at each of the putative nucleosome is also a source of locus-wise epigenetic variation. The primary source of phenotypic variability, within and between populations of a plant species, are the differences in DNA sequences arising from mutations (frameshift, transition, transversion

*For correspondence. E-mail: sushil2000_01@yahoo.co.in.

Keywords. cytosine methylation; DNA methylation mechanisms; DNA demethylation mechanisms; Darwinian-cum-Lamarckian evolution; epialleles; epigenetic modifications; genetic recombination; heritable induced defence; mutational hotspots; transgenerational inheritance.

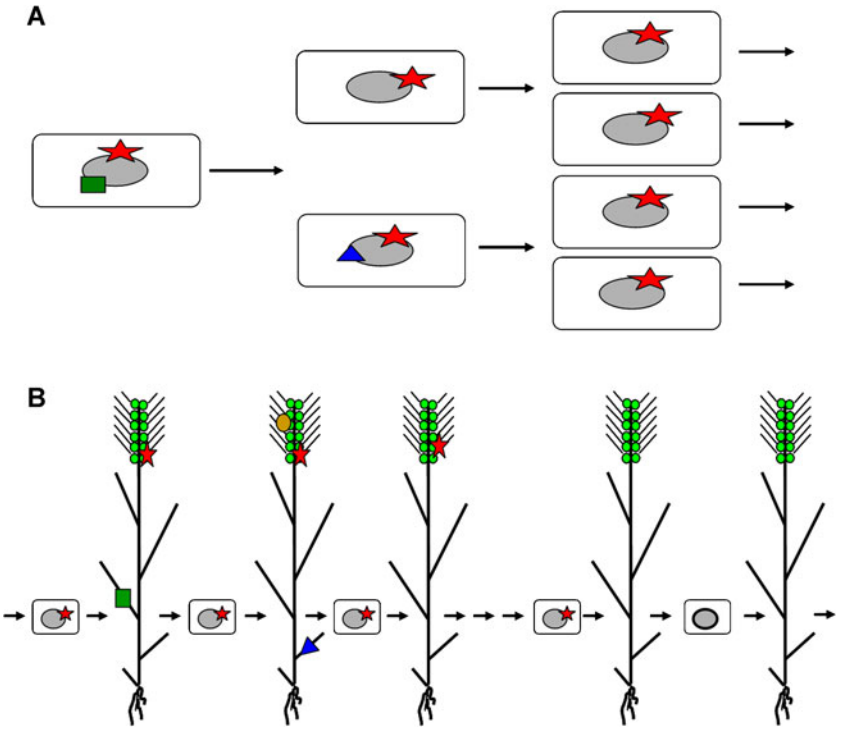

Figure 1. Variation in the heritability of epigenetic marks (changes) in mitosis and meiosis. While some of the epigenetic changes that are transient are not passed to progeny cells (depicted as green square and blue triangle), the stable ones are mitotically inheritable (transgenerational). The meiotically heritable epigenetic changes are passed on to progeny (depicted as red star) and are heritable for several to many generations without the need of original stimulus, until they are lost or erased on account of genetic change, spontaneously for unknown reason(s) or for reasons of exposure to environmental conditions distinct from those that led to the epigenetic change in the first instance (depicted as red star). The mitotically heritable changes that do not pass through meiosis (depicted as green square, blue triangle and yellow circle) are lost since they are not passed to the progeny.

Table 1. Comparison of mutations due to changes in DNA base sequence on one hand and DNA cytosine base modifications (epimutations) on the other hand and features of their inheritance in plants.

Allele(s) arising from base sequence changes (normal mutations)	Allele(s) arising from cytosine base methylations (DNA methylation led epimutations)
Origin due to errors in DNA replication, spontaneous and consequent to DNA damage caused by a variety of agents, including high temperature and ultraviolet light and additions and deletions resulting from movement of transposable elements; frequency low	Result from loss or gain of DNA methylation, in response to exposure to environmental stresses; frequency high
Phenotypic change due to alteration or loss of gene function(s) or alteration of gene networking in developmental processes and/or in response to environmental stimuli	Alteration in gene networking in developmental processes and/or in response to environmental changes
Stable through mitotic divisions	Relatively less stable (unstable)
Invariably transmitted through gametes	Due to their repair, the changes may not be passed on to gametes
Inheritance is regular (non-Lamarckian) and is persistent	Phenotypic change acquired due to changes in DNA methylation may persist only for a few generations (Lamarckian inheritance plausible), unless favourable base sequence changes in genes of importance for adaptation to a given environmental stress targeted by the pruning of methylated cytosines, get selected

mutations and various types of extended addition–deletion mutations). Sites of cytosine methylations are themselves hotspots for occurrence of mutations. The cytosine methylation and histone modification marks amplify the genetic variation manifold epigenetically (table 1). Whereas populations may share the genetically programmed epigenetic patterns in gene promoters and/or gene bodies based on evolutionarily selected developmental needs of gene regulatory networks, bulk of intrapopulation and interpopulation epigenetic pattern variation is because of the differential induction by abiotic and biotic stresses. The mechanisms that impart cytosine methylation and histone modifications are genetically inherited in plants. They became parts of plant's genetic programme to impart them fitness, since plants are sessile and face drastic changes of seasons and extreme weather, water and nutrition availability in their environment. Epigenetic genome variation is known in both eukaryotes and prokaryotes (Johnson and Coghill 1925; Arber and Linn 1969; Lyko *et al.* 2000) and among eukaryotes in fungi, animals and plants (Holliday and Pugh 1975; Maloisel and Rossignol 1998; Law and Jacobsen 2010; Deal and Henikoff 2011). There are differences in the use of DNA methylation among different classes of eukaryotes. Transposons and repeats are methylated in fungi, genes and not transposons are methylated in lower invertebrates, and plants and other animals methylate genes, repeats and transposons (Saze and Kakutani 2007; Feng *et al.* 2010; Zemach *et al.* 2010). Histone modification and cytosine methylation are examples of epigenetic variation that exists in mammals as well as in plants (Bernstein *et al.* 2006; Law and Jacobsen 2010; He *et al.* 2011; Lauria and Rossi 2011; Margueron and Reinberg 2011). The cytosine methylation process is more complex in plants (for the purposes of this review, from here onwards the term plants means flowering plants/angiosperms) than in mammals. Forward and reverse genetic analyses of epigenetic processes are being actively pursued in several model plants: *Arabidopsis thaliana* (eudicot), *Zea mays* and *Oryza sativa* (monocots). This review covers aspects of DNA methylation-induced epigenetic variation. It covers types of epigenetic marks and interplay of cytosine methylation and histone modification mechanisms, establishment, maintenance and loss of DNA methylation, origin of epialleles and paramutation, transposon silencing, high mutational rate due to loss of methylated cytosines, evolutionary consequences of epigenetic variation and possible applications, of heritable epigenetic marks.

Epigenetic modifications and interplay in the establishment of DNA methylation and histone modification marks

Both histone modifications and DNA methylation are important in maintaining genomic integrity and regulation of gene expression (Lauria and Rossi 2011). Of the four bases in DNA (A, adenine; T, thymine; G, guanine; C, cytosine), adenine and cytosine undergo modifications. Adenine modifications have been relatively much less investigated than cytosine methylation. Cytosine residues in plant nuclear genomes are methylated in CG, CHG and CHH (where H = A, T or C) contexts; cytosine in each of the three contexts is methylated by a distinct methyltransferase (table 2; figure 2). A general property of cytosine methylation marks is repression over the gene(s) at the sites of occurrence. Chromatin exists in the form of a string of nucleosomes connected by DNA. About 150 bp DNA is tightly coiled around each nucleosome which consists of a pair, each of four core

Table 2. Common types of epigenetic marks and proteins/enzymes that establish them in the model plant, *Arabidopsis thaliana.*

Epigenetic mark	Protein(s)/enzyme(s) that establish it on chromosomes	Location of mark(s)
Cytosine methylation[a]		
CG	METHYLTRANSFERASE 1 (MET1)	Genes, transposable elements (TE) and repeat sequences (RS)
CHG	CHROMOMETHYLASE 3 (CMT3)	TE and RS
CHH	DOMAIN REARRANGED METHYLTRANSFERASE 2 (DRM2)	TE and RS
Histone methylation[b]		
H3K4me1/me2/me3	ARABIDOPSIS HOMOLOGUE OF TRITHORAX (ATX1 to ATX5); ARABIDOPSIS TRITHORAX RELATED (ATXR1 to ATXR7) and ASH1 HOMOLOGUE (ASHH1 to ASHH14)	Genes
H3K27me3	Polycomb group (PcG)	Genes
H3K9me2	KRYPTONITE (KYP); SUVH5 and SUVH6	Heterochromatin (TE and RS)

[a]H: A, T or C; [b]H3, histone H3; K, lysine amino acid at 4th, 9th or 27th position in H3 N-terminal tail; me1, me2 or me3, me3 and me2, respectively: addition of 1, 2 or 3 methyl group(s) (Pontvianne *et al.* 2010).

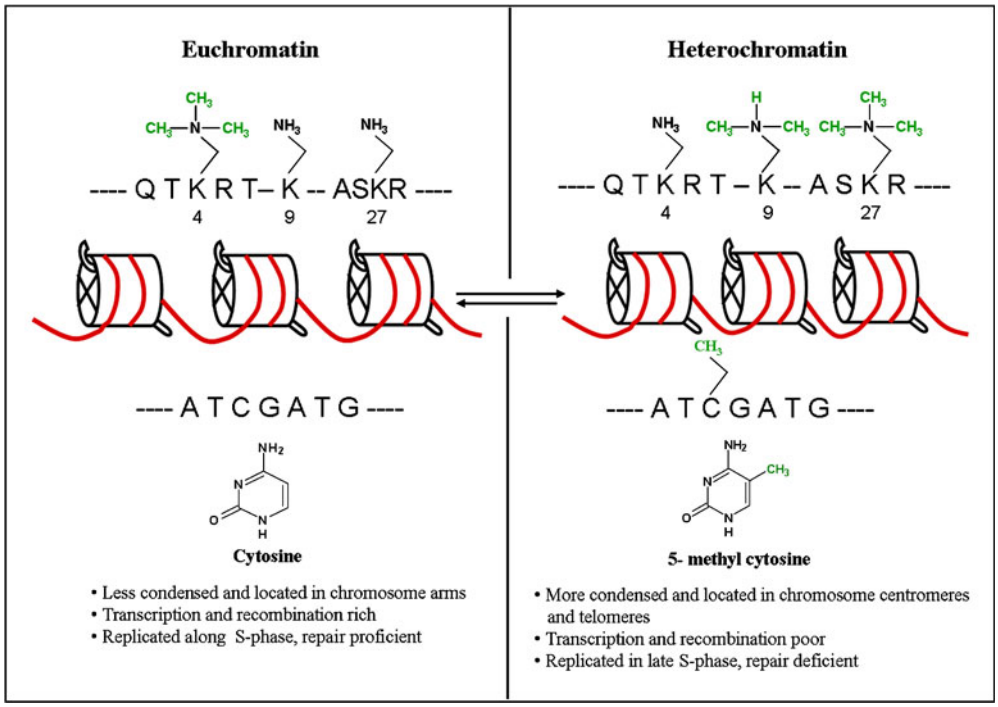

Figure 2. Cytosine and/or histone methylation related and other differences between euchromatin and heterochromatin (adapted after Bernstein *et al.* 2006). Cytosine methylation is a significant DNA methylation and occurs in CG, CHG (H = A, T, C or G) and CHH (H = A, T or C) contexts in plants. There are numerous kinds of modifications in histones (table 3). In the euchromatin, cytosine in DNA is shown to be unmethylated and lysine at position 4 in H3 histone has methylation, which results in activating effect on transcription. In the heterochromatin, cytosine is methylated at its 5th position and the lysines at position 9 and at position 27 of histone H3 are also methylated, which cause repressive effect on transcription. Nucleosomes are condensed in heterochromatin relative to chromatin.

histones H2A, H2B, H3 and H4. Histones of nucleosomes undergo covalent modifications at different aminoacid positions. The modifications that occur in lysine and arginine residues, especially those in the N-terminal tails of histones, are relatively more important epigenetic marks (Berger 2007; Kouzarides 2007; Deal and Henikoff 2011). In lower eukaryotes and mammals, histones have been found to undergo acetylation, methylation, phosphorylation, ubiquitination, sumoylation, ADP ribosylation, proline isomerization and a variety of other modifications (table 3). The structural and functional features of modified histones are highly conserved across eukaryotes. Histone modifications are less studied in angiosperms. However, it has been found (table 2; figure 2) that methylations on lysine at fourth position on histone 3 (H3K4me) and acetylation on lysine at ninth position on H3 (H3K9ac) are conducive for gene expression, trimethylation of lysine at 27th position on H3 (H3K27me3) is repressive for gene expression and dimethylation on ninth lysine in H3 (H3K9me2) leads to heterochromatization (Fuchs *et al.* 2006; Zhang *et al.* 2009; Huff and Zilberman 2012) (table 3). Exchange and loss of histones have been observed to affect gene expression and genome integrity in plants (Mizuguchi *et al.* 2004; Wu *et al.* 2005; Zhang 2008; Kumar and Wigge 2010; Papamichos-Chronakis *et al.* 2011). Chromatin is roughly divisible into two types of domains: gene rich, loosely packed euchromatin and gene deficient, densely packed heterochromatin (Bernstein *et al.* 2006; Rangwala and Richards 2007; Roudier *et al.* 2011) (figure 2). Heterochromatin comprises largely of repeat sequences, pseudogenes and transposable elements, especially retrotransposons. Cytosine methylation marks and histone modification marks keep the genetic elements of heterochromatin silenced (Rabinowicz *et al.* 2003; Kakutani *et al.* 2004; Lippman *et al.* 2004; Wassenegger 2005; Topp and Dawe 2006; Grewal and Elgin 2007; Morris and Moazed 2007; Saze and Kakutani 2011). Heterochromatin is important in centromere functioning at cell divisions and in the three dimensional interchromosomal relationships in the nucleus (Henikoff *et al.* 2001; Nagaki *et al.* 2003; Calonji and Sung 2006; Kumar 2007; Luo and Lam 2010).

Several associations between cytosine methylation and histone modifications have been revealed in *A. thaliana*. A correlation has been observed between the sites of CHG methylation in DNA and histone H3 methylation in lysine 9 and lysine 27) in chromatin. Simultaneous formation of methylation marks at lysines 9 and 27 in H3 by the SUVH4 or KRYPTONITE (KYP) histone methyltransferase allows recruitment of CHROMOMETHYLASE 3 (CMT3) cytosine methyltransferase at the CHG site(s) in DNA (Jackson *et al.* 2002;

Table 3. Reversible histone modifications that cause structural changes and/or affect recruitment of effector proteins on chromatin.

Kind of modification	Transcriptionally relevant site(s) of modification	Effect of modification on chromatin and transcription from it and DNA repair on it
Phosphorylation of cysteine/threonine in histone (ph S/T)[a]	H3 (3, 10, 28), H3A and H3B	Repression of transcription and DNA repair and chromatin condensation
Acetylation of lysine in histone (ac K)[a]	H3 (9, 14, 18, 56), H4 (5, 8, 13, 16), H2A and H2B	Transcription activation
Methylation of arginine (me R)[b]	H3 (17, 23) and H4 (3)	Do
Methylation of lysine (me K)[b′]	H3 (4, 36, 79)	Activation of transcription and DNA repair
Methylation of lysine (me K)[b′]	H3 (9, 27) and H4 (20)	Repression of transcription
Sumoylation of lysine (su K)[c]	H2B (6/7) and H2A (126)	Do
Isomerization of proline (isom P)	H3 (30, 38)	Transcription activation
Ubiquitination of lysine (ub K)[d]	H2B (120)	Activation of transcription and DNA repair
Ubiquitination of lysine (ub K)[d]	H2A (119)	Repression of transcription
ADP ribosylation (ar E)	H2B	Do
Deimination (R > cit)	H3, H4	Do

[a, b, b′] These modifications involve small chemical groups; [c,d] these modifications involve large moieties which are themselves 66% the size of histones they modify and may lead to drastic changes in chromatin structure, [b]methylations can be mono-, di- or tri- on one lysine side chain with different effects on chromatin. Based on the descriptions of Berger (2007), Kouzarides (2007) and Deal and Henikoff (2011).

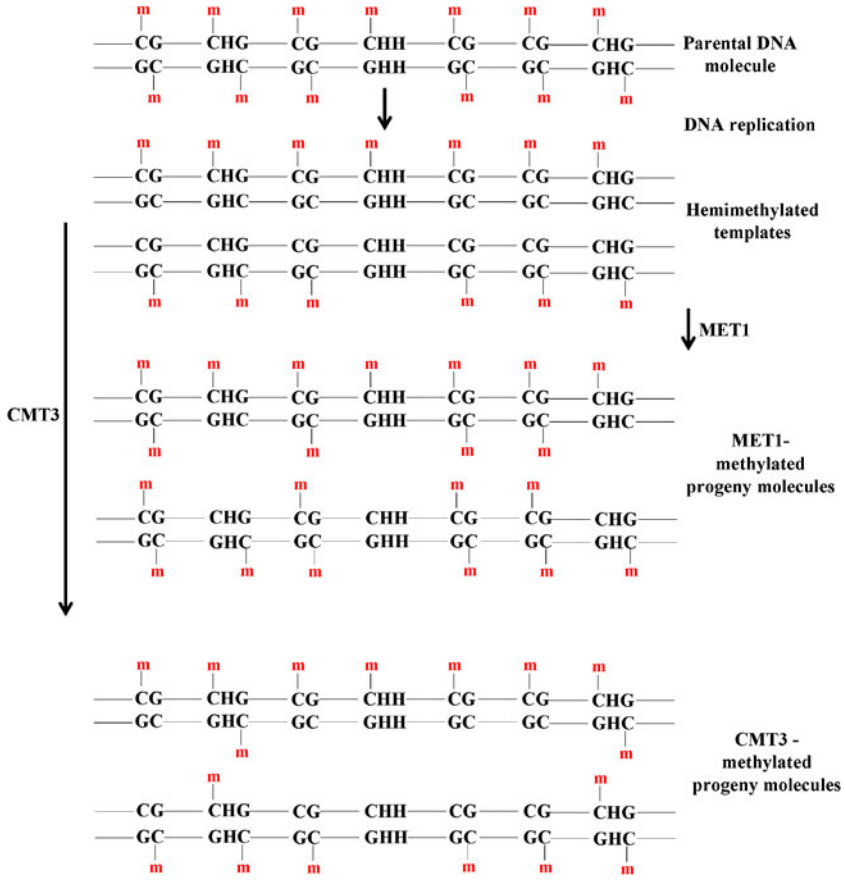

Figure 3. Diagramme depicting the maintenance of cytosine methylation marks in replicating DNA in CG and CHG contexts. MET1 cytosine methylase carries out cytosine methylation in the nascent DNA strand in CG context. Hemimethylated DNA strands are cytosine methylated in CHG context by the CMT3 methylase. Cytosines in CHH context are not methylated by MET1 and CMT3 enzymes. These are methylated by the RdDM process. Whereas in the CMT pathway, CMT3 is guided to the target site by histone methylation through KRYPTONITE (KYP). In the MET1 pathway, Met1 is directed to the target by HISTONE DEACETYLASE (HDA).

Lindroth *et al.* 2004; Johnson *et al.* 2007). SUVH2 and SUVH9 histone methylases are essential for methylation at CHH sites (Johnson *et al.* 2008). The CHH methylation in DNA is promoted by JMJ14, a histone demethylase of H3K4me3 in chromatin (Searle *et al.* 2010). Symmetric DNA methylation at CG sites is induced in chromatin sites where H4 histone(s) are deacetylated by the HDA6 histone deacetylase (Aufsatz *et al.* 2002; To *et al.* 2011a, b).

DNA methylation

The DNA methylation process refers to the addition of a methyl group to the fifth position of carbon of cytosine base in DNA (figure 2; table 2). In plants (much of the information has accrued from forward and reverse genetic analyses in the model plant *A. thaliana*), there are two mechanisms for DNA methylation: *de novo* methylation and maintenance methylation (Chan *et al.* 2005; Saze *et al.* 2012). The DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) catalysed process methylates cytosines in CG, CHG and CHH contexts and also maintains methylation at Cs in the asymmetric CHH elements, in cell divisions (Cao *et al.* 2003; Pontes *et al.* 2006). METHYLTRANSFERASE 1 (MET1) (Aufsatz *et al.* 2004) and CHROMOMETHYLTRANSFERASE 3 (CMT3) (Lindroth *et al.* 2001), respectively, maintain cytosine methylation at the symmetric CG and CHG sequence elements in cell divisions

Table 4. Genes/factors that mediate and maintain RNA directed DNA methylation at cytosines in CHH, CG and CHG contexts in *Arabidopsis thaliana*[a].

Gene/product	Activity/function	Reference(s)
POLYMERASE IV (POL IV)	Transcribes the target sequence	Herr *et al.* (2005); Onodera *et al.* (2005); Zhang *et al.* (2007)
CLASSY 1 (CLSY1)	SNF2-like chromatin modelling factor	Kanno *et al.* (2004); Smith *et al.* (2007)
RNA DEPENDENT RNA POLYMERASE 2 (RDR2)	Single stranded RNA (ssRNA) dependent RNA polymerase	Xie *et al.* (2004); Lu *et al.* (2006); Law *et al.* (2011)
DICER-LIKE 3 (DCL3)	Endonuclease that cleaves double stranded RNA (dsRNA) into 24-nucleotide long fragments or siRNAs	Xie *et al.* (2004); Henderson *et al.* (2006)
HUA ENHANCER 1 (HEN1)	Nuclear methyltransferase that stabilizes siRNAs by 3′-terminal ribose methylation	Yu *et al.* (2005); Yang *et al.* (2006); He *et al.* (2009)
ARGONAUTE4 (AGO4) or *AGO6*	Binds the stabilized ss siRNA to form the silencing effector complex	Zilberman *et al.* (2003, 2004); Qi *et al.* (2006); Zhang *et al.* (2007)
POLYMERASE V (POL V)	Generates scaffold non-coding RNA transcripts from the target region	Wierzbicki *et al.* (2008, 2009); Ream *et al.* (2009)
KOW-DOMAIN CONTAINING TRANSCRIPTION FACTOR 1 (KTF1)	RNA binding protein that confines *AGO4* effector complex to nascent *POL V* non-coding RNA transcript	He *et al.* (2009); Huang *et al.* (2009); Bies-Etheve *et al.* (2009)
DEFECTIVE IN MERISTEM SILENCING3 (DMS3)	Hinge domain containing protein that helps in stabilizing siRNA-Pol V transcript RNA–RNA pairing	Kanno *et al.* (2008); Ausin *et al.* (2009); Greenberg *et al.* (2011)
INVOLVED IN DE NOVO 2 (IDN2)	Effector protein that has XS domain which is known to bind to dsDNA; it may stabilize RNA–RNA pairing	Ausin *et al.* (2009); Zheng *et al.* (2010); Greenberg *et al.* (2011)
RNA DIRECTED DNA METHYLATION PROTEIN 1 (RDM1)	A novel protein that is a necessary part of DDR complex that facilitates *POLV* transcription	Law *et al.* (2010)
DEFECTIVE IN RNA DIRECTED DNA METHYLATION (DRD1)	SNF2 chromatin remodelling protein required for siRNA alignment with DNA target sequence	Kanno *et al.* (2004); Zhang *et al.* (2007)
DEFECTIVE IN MERISTEM SILENCING 4 (DMS4)	Transcription factor required for *POLV* or *POL II* activity at the target loci	He *et al.* (2009); Kanno *et al.* (2010); Greenberg *et al.* (2011)
HISTONE DEACETYLASE 6 (HDA6)	Essential for RNA directed DNA methylation	He *et al.* (2009); Greenberg *et al.* (2011); Aufsatz *et al.* (2002)
SU(VAR)3-9 HOMOLOG2 (SUVH2) and *SUVH9*	SRA-SET proteins, essential for retaining DRM2 at the target loci	Johnson *et al.* (2008); Ausin *et al.* (2009); Zheng *et al.* (2010); Greenberg *et al.* (2011)
DOMAIN REARRANGED METHYLTRANSFERASE (DRM2)	Principal *de novo* DNA methylation enzyme for cytosine methylation at CHH, CHG and CG contexts	Cao *et al.* (2003); Aufsatz *et al.* (2004)
DRM3	Mutant form of *DRM2* that stimulates *DRM2* activity	Henderson *et al.* (2010)

[a]The roles of various factors are diagrammatically shown in figure 4.

(Woo *et al.* 2007; Woo and Richards 2008) (figure 3). Whereas the *de novo* methylation process is well characterized, maintenance DNA methylation processes, involving semiconservative propagation of cytosine methylation marks, are less understood.

The *de novo* DNA methylation process is also known as RNA-directed DNA methylation (RdDM), because the putative methylation sites are targeted via the homologies in small interfering RNA (siRNA) (Law and Jacobsen 2010; Haag and Pikaard 2011; He *et al.* 2011; Kanno and Habu 2011). Angiosperms have RdDM conserved in them. RdDM comprises of three steps, biogenesis of siRNA, production of scaffold RNA and targeting of putative methylation sites by DRM2 with aid from a large number of proteins and pairing between siRNA and scaffold RNA (table 4; figure 4).

The siRNAs are short (typically of 24 nucleotides (nt)) generated from noncoding components of the genome. They are formed by the RNA interference pathway. All plants have the DNA dependent RNA polymerases I, II and III (pol I, pol II and pol III). In angiosperms, pol IV and pol V participate exclusively in RdDM. Of the 12 subunits they contain, two are common between them, six are shared with pol II and rest are specific (Ream *et al.* 2009; Huang *et al.* 2009; Law *et al.* 2011). Pol IV generates a single stranded RNA (ssRNA) transcript of the target region by using the methylated DNA as template (Herr *et al.* 2005; Pontes *et al.* 2006; Zhang *et al.* 2007; Wierzbicki *et al.* 2008). RNA DEPENDENT RNA POLYMERASE 2 (RDR2) in interaction with pol IV converts ssRNA immediately into double stranded RNA (dsRNA). The dsRNA serves as precursor for 24 nt siRNA obtained by chopping of dsRNA by DICER-LIKE 3 (DCL3) (Xie *et al.* 2004; Matzke *et al.* 2007; Pikaard *et al.* 2008; Law *et al.* 2010). The siRNAs are stabilized/matured by their 3′-terminal ribose methylation by HUA ENHANCER 1 (HEN1). The mature siRNAs are loaded on to ARGONAUTE 4 (AGO4) or AGO6 (Li *et al.* 2006; Pontes *et al.* 2006; Zheng *et al.* 2007; Mi *et al.* 2008). The RDR2 and pol IV activities are replaceable by pol II in the case of inverted repeat sequences (Zhang *et al.* 2007; Pikaard *et al.* 2008). CLASSY1 (CLSY1) a SWI2/SNF2-like chromatin remodelling factor functions together with RDR2 and pol IV (Smith *et al.* 2007). The transcription factor RNA DIRECTED DNA METHYLATION (RDM4, also known as DMS4) serves as an essential regulatory factor for the activities of both pol II and pol V (He *et al.* 2009; Kanno *et al.* 2010). Both pol II and pol V can generate scaffold RNAs for

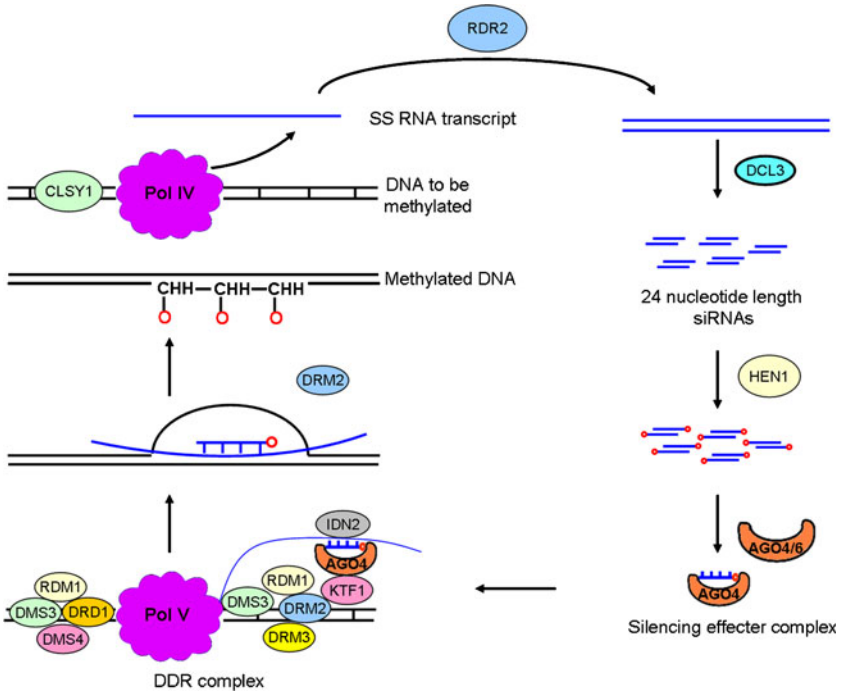

Figure 4. Diagrammatic representation of *de novo* RNA directed methylation (RdDM) of cytosines in CHH, CHG or CG contexts. Aided by CLYS1, POL IV transcribes a DNA sequence to generate a single stranded RNA (ssRNA). The ssRNA is converted into double stranded RNA (dsRNA) by RDR2. The dsRNA is diced into 24-nt siRNAs by DCL3. Thereafter 3′ ends of siRNAs are methylated by HEN1. Any one of the modified strands of siRNA is loaded on to AGO4 or AGO6 to form the silencing effector complex. DMS3, DMS4, DRD1 and RDM1 become a part of the DDR complex that facilitates transcription of the RdDM target DNA by pol V. Transcription of intergenic noncoding region by pol V generates a noncoding RNA that serves as scaffold RNA. This RNA via its complementarity to siRNA recruits AGO4/6 to chromatin. The dsRNA formed by siRNA-scaffold RNA gets stabilized by the binding of KTF1 and IDN2 to the hybrid. Via intermediation of RDM1 and/or SUVH2/9, DRM2 is recruited to methylate the target DNA.

RdDM (El-Shami *et al.* 2007; Pikaard *et al.* 2008; Wierzbicki *et al.* 2008, 2009; Zheng *et al.* 2009). To be effective, the scaffold RNAs get generated from the noncoding sequences lying adjacent to the siRNA generating loci (Wierzbicki *et al.* 2008, 2009; Zheng *et al.* 2009). Pol II generates scaffold RNAs especially at the intergenic loci of low copy numbers. For scaffold RNA synthesis, pol V requires DEFECTIVE, IN RNA-DIRECTED DNA METHYLATION 1 (DRD1, a chromatin modelling protein), RDM1 and DEFECTIVE IN MERISTEM SILENCING 3 (DMS3) (Kanno *et al.* 2004, 2008; Wierzbicki *et al.* 2008, 2009; Zheng *et al.* 2009; Gao *et al.* 2010; Law *et al.* 2010) and pol II requires DRD1 only (Zheng *et al.* 2009). The pol V and pol II transcripts may also serve as templates for RDR2.

DRM2 is guided to the target site by a complex of interacting factors. siRNAs borne on AGO4 and AGO6 pair with complementary sequences in target DNA or scaffold RNAs and form the core of the complex. The complex includes pol V, pol II, DRD1, DMS3, RDM1, KOW-DOMAIN CONTAINING TRANSCRIPTION FACTOR 1 (KTF1), INVOLVED IN DE NOVO 2 (IDN2), DRM3 (a mutant form of DRM2), HISTONE DEACETYLASE 6 (HDA6) and SU(VAR) 3–9 HOMOLOG 2 (SUVH2) and SUVH9 (table 4). Among the members of the complex, RDM1 has ability to be interactive with both AGO4 and DRM2 (Gao *et al.* 2010; Law *et al.* 2010). KTF1, an RNA binding protein, is thought to teether AGO4 to scaffold RNA (Wierzbicki *et al.* 2008, 2009; Bies-Etheve *et al.* 2009; He *et al.* 2009). IDN2 which has an XS domain is able to stabilize the siRNA-scaffold RNA pairing (Ausin *et al.* 2009; Zheng and Chen 2011). SUVH 2/9 cooperates with DRM2 in the elaboration of latter's DNA methylation activity by retaining DRM2 at the target site (Johnson *et al.* 2008). DRM3 is stimulatory for the DNA methylating activity of DRM2 (Henderson *et al.* 2010).

Recently, it has been observed that pol IV and pol V led RdDM accounts for DRM2 driven *de novo* DNA methylation at about 50% of the CHH positions in the *Arabidopsis* genome. Some other methyltransferase(s) may be involved for methylation of cytosines at CHH sites in the pericentromeric heterochromatin like DNA sequences (Wierzbicki *et al.* 2012). RdDM is active in all tissues and cell types in vegetative and reproductive phases of plants. It has critical role in resetting of the epigenetic marks in nascent embryo after fertilization. In this process in the embryo sac, the endosperm and companion cells play an essential role by transferring the products of siRNA biogenesis in them. Restoration of methylation marks lost in *met1* mutants after retransfer of *MET1* occurs by RdDM and random and transcription based *de novo* methylation activity of *MET1* (Zubko *et al.* 2012). CHG DNA methylation maintenance takes place by a selfreinforcing loop that involves histone modification. In this process, CMT3 binds to H3K9me2 whose modification is catalysed by KYP (histone methyltransferase) (Jackson *et al.* 2002; Lindroth *et al.* 2004). Similarly, the SRA domain of KYP binds to methyl cytosine in CHG site in DNA (Johnson *et al.* 2007). These two interactions ensure that CHG methylation in DNA and dimethylation in lysine 9 of H3 histone will get propagated indefinitely. This process of CHG maintenance does not cover CHG sites in genes. Heterochromatin methylation of cytosines at CG site by MET1 is aided by VIM1 (VARIANT IN METHYLATION 1), a methyl cytosine binding protein. VIM1 associates with histone proteins via its SRA domain, providing an interface between DNA methylases and histone proteins. VIM1 and its paralogs are

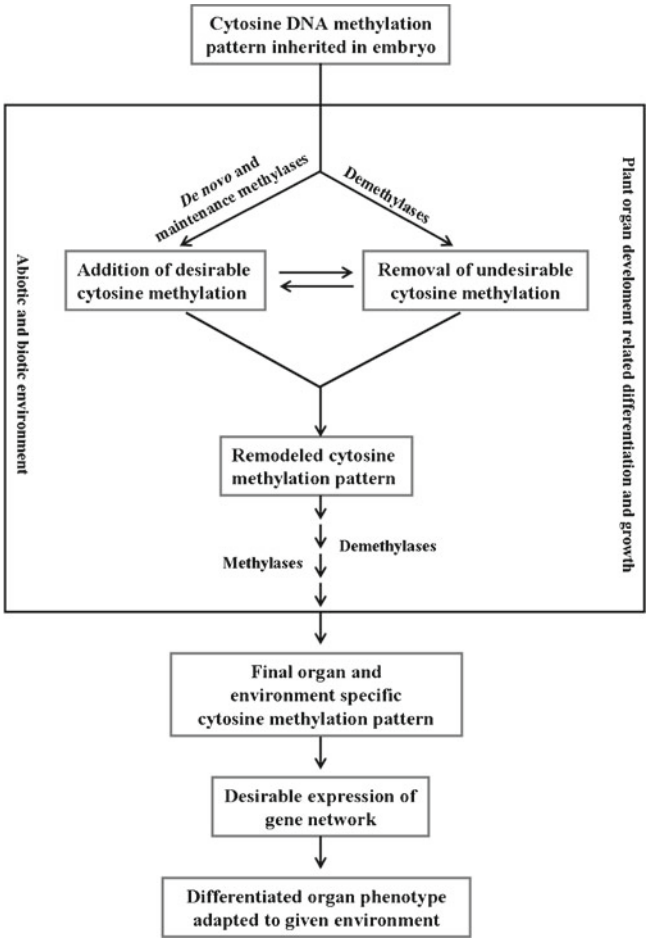

Figure 5. Diagrammatic representation of the processes for obtaining cytosine DNA methylation suitable for organ development under specific environmental conditions. The cytosine methylation pattern of the embryo is remodelled in organs according to prevailing biotic and abiotic conditions. The remodelling involves pruning undesirable methylations, establishment of desirable new methylations and maintenance of desirable cytosine methylations. This process continues throughout the life of the organ. Genes are expressed organ-wise according to the genotype, environment and methylation pattern. The pruning of cytosine methylations occurs by the action of DME, ROS1, DML1 and DML2 methylases. *De novo* cytosine methylation is achieved by DDR complex of which DRM2 methylase is a part. Methylations in the context of CG and CHG are maintained by the activities of MET1 and CMT3, respectively.

perhaps required for the recruitment of MET1 to hemimethylated CG sites in nascent DNA strand (Woo *et al.* 2007; Woo and Richards 2008). Details of how exactly CG and CHG methylations are maintained in genes remain to be understood.

DNA demethylation

Normally, the cytosine or DNA methylation patterns once established are maintained through mitotic cell divisions. Cytosine methylation patterns arise by antagonistic actions of DNA methylases and DNA demethylases. DNA demethylation is perhaps a corrective process which on one hand removes the promiscuous methylations and on the other activates specific genes or prepares DNA for resetting of methylation marks in response to environmental perturbation or requirements of cell differentiation during development (figure 5).

Demethylation in DNA can occur passively or actively (Zhu 2009). Passive demethylation takes place during DNA replication. The newly synthesized strand during DNA replication in a cell cycle remains unmethylated by the inaction of maintenance DNA methylases MET1 and CMT3 (Zhu 2009). There is progressive loss of DNA methylation if MET1 and CMT3 remain inactive during several cell divisions. The active DNA methylation occurs by a base excision repair pathway (table 5; figure 6). This process is initiated by DNA demethylase. This kind of enzyme is bifunctional by possessing glycosylase and lyase activities (Choi *et al.* 2002; Gong *et al.* 2002). DNA methylase first removes the 5-methyl cytosine as a free base and then cleaves the DNA phosphodiester backbone at the site of methyl cytosine removal by β,δ-elimination. The product has a single nucleotide gap flanked by 3′ and 5′ phosphate termini (Bhutani *et al.* 2011). A DNA 3′ phosphatase called ZDP removes the blocking 3′ phosphate (Martinez-Macias *et al.* 2012). The gap is now filled with nonmethylated cytosine by DNA polymerizing and DNA ligating steps (Agius *et al.* 2006; Bhutani *et al.* 2011). Four DNA demethylases are known in *Arabidopsis*: REPRESSOR OF SILENCING 1 (ROS1), DEMETER (DME), DEMETER-LIKE (DML2) and DML3 (Choi *et al.* 2002; Gong *et al.* 2002; Ortega-Galisteo *et al.* 2008) (table 5). The DNA demethylases find their target either by sliding on the DNA molecule bearing the target (Ponferrada-Marin *et al.* 2012) or they interact with ROS3 to reach the target site. ROS3 has ability to bind to siRNAs. The homology between siRNA and target DNA sequence may facilitate finding of target by DNA methylase interaction with siRNA loaded ROS3 (Zheng *et al.* 2008). *INCREASE IN BONSAI METHYLATION 1 (IBM1)* gene product, a jmj (Jumonji C) domain demethylase of H3K9me, is also involved in pruning of methylation from cytosines at non-CG sites in many repeated heterochromatin loci in *A. thaliana* (Saze *et al.* 2008). This process is antagonistic to H3K9me directed cytosine methylation by CMT3 at non-CG sites.

The passive and active DNA methylation processes may function independently or in combination (Morales-Ruiz *et al.* 2006). The DME, ROS1, DML2 and DML3 demethylate DNA independent of its replication and function redundantly (Choi *et al.* 2002; Gong *et al.* 2002; Penterman *et al.* 2007). There does not appear to be any tissue specificity for passive demethylation or active demethylation dependent on ROS1, DME, DML2 or DML3 (Gehring *et al.* 2006; Penterman *et al.* 2007; Zhu *et al.* 2007; Lister *et al.* 2008).

Table 5. Genes/factors involved in the active DNA demethylation in *A. thaliana.*

Gene/product	Activity/function	Reference(s)
DEMETER1 (*DME1*)	DNA glycosylase activity at the cytosine methylated sites in CG, CHG and CHH contexts	Choi *et al.* (2002)
DME-LIKE2 (*DML2*)	As above	Ortega-Galisteo *et al.* (2008)
(*DML3*)	As above	As above
REPRESSOR OF SILENCING1 (*ROS1*)	As above	Gong *et al.* (2002)
DNA 3′ PHOSPHATASE (ZDP)	In interaction with ROS1, the DNA phosphatase ZDP removes 3′ phosphate from the single nucleotide gap flanked by 3′ and 5′ phosphate termini, by acting downstream of ROS1	Martinez-Macias *et al.* (2012)
ROS3	Single strand RNA binding protein that assists in pruning of C-methylation in CG, CHG and CHH contexts	Zheng *et al.* (2008)
KRYPTONITE (*KYP*)	HISTONE H3K9 METHYLTRANSFERASE or SUVH4 KYP that assits in removal of cytosine-methylation in CHG contexts	Saze *et al.* (2008); Miura *et al.* (2009); Inagaki *et al.* (2010)
INCREASE IN BONSAI METHYLATION (IBM)	HISTONE H3K9 DEMETHYLTRANSFERASE that assists in removal of cytosine-methylation in CHG contexts	Saze *et al.* (2008)

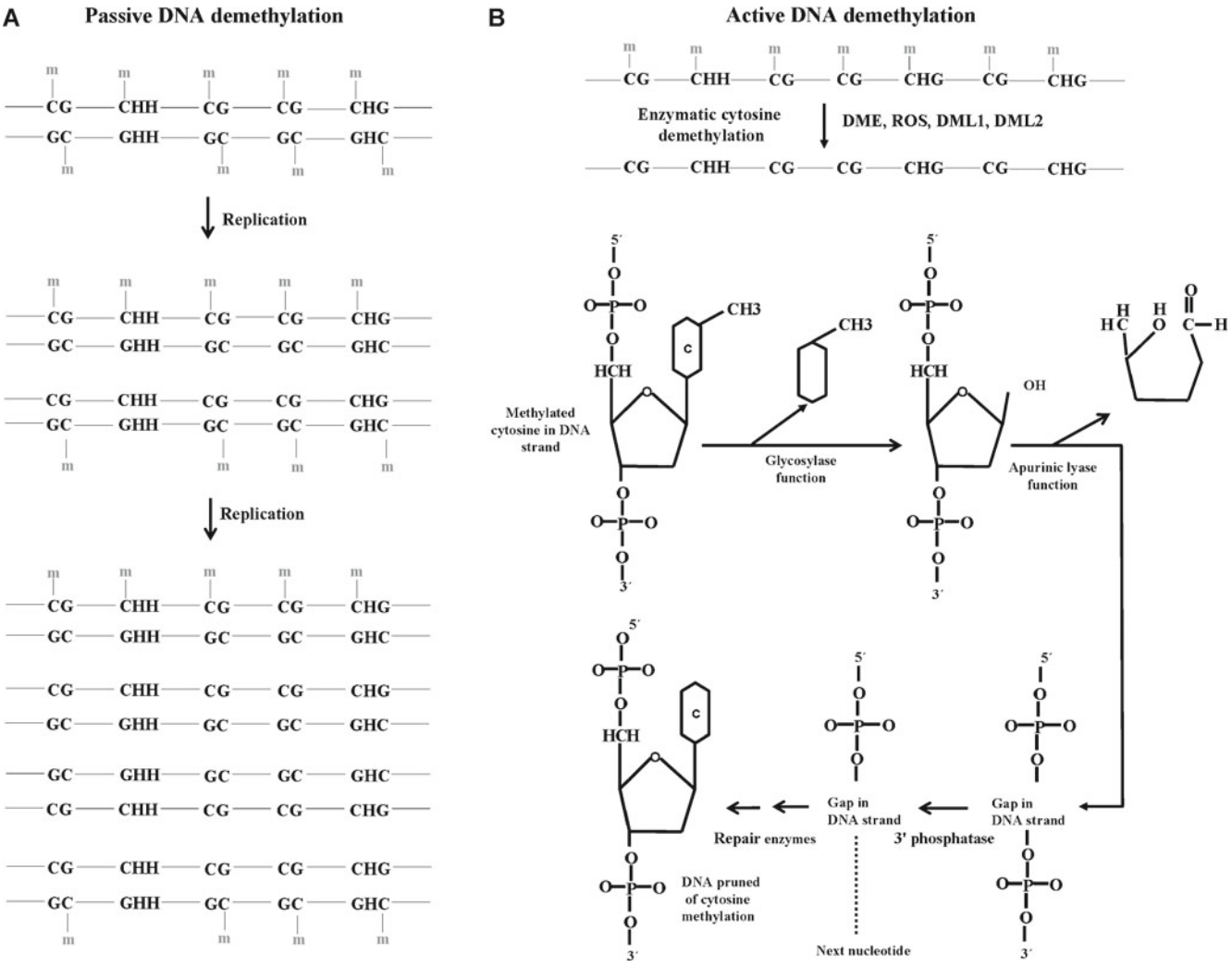

Figure 6. Passive and active DNA demethylation. (A) A DNA molecule bearing nine methylated cytosines, six in CG, two in CHG and one in CHH contexts, is shown. After two rounds of replication, in the absence of fresh C-methylation, only 9/36 cytosines are seen methylated, on account of passive DNA demethylation; (B) a single strand of DNA molecule bearing seven methylated cytosines is shown, four in CG, two in CHG and one in CHH contexts. The action of ROS1, DME, DML1 and/or DML2 is shown to have pruned the DNA molecule of methylation. The products of *ROS1*, *DME, DML2* and *DML3* being bifunctional DNA glycosylases, their demethylation occurs by a base excision repair pathway which is diagrammed. First, the 5-methylcytosine base is removed, next, backbone is cleaved at the abasic site. The gap so created is flanked by 3′-phosphate and 5′-phosphate termini. The 3′-phosphate from the gap is removed by a 3′-phosphatase ZDP enzyme. Subsequently, the unblocked gap is filled by an unmethylated cytosine nucleotide, aided by DNA polymerase and DNA ligase enzymes of as yet unknown identity in *A. thaliana*. Errors in the process may be mutagenic.

Imprinting in companion cells and endosperm and resetting of methylation marks in male and female gametes and embryo

Diploid sporophyte and haploid gametophyte generations alternate in the life cycle of plants. Sporophyte ($2n$) develops from the fusion product of haploid (n) male and female gametes. Late in its life, sporophyte produces flowers in which the meiotic products of anthers are pollen and that of ovary are embryo sacs. Pollen consists of a vegetative cell and two sperm cells which are the male gametes. The embryo sac has a binucleate gamete as the central cell, a uninucleate gamete as the egg cell and five companion cells (two synergids and three antipodals). Double fertilization occurs such that a sperm fertilizes the egg cell and the second one the central cell. The fertilized egg cell or zygote grows into an embryo, an early stage sporophyte. The fertilized central cell develops into endosperm, which nurses the embryo (figure 7). Endosperm subsequently dies in eudicots. In monocotylednous plants, endosperm is persistent and grows into an organ of the seed. The DNA methylation patterns fluctuate through sporophyte–gametophyte precursor

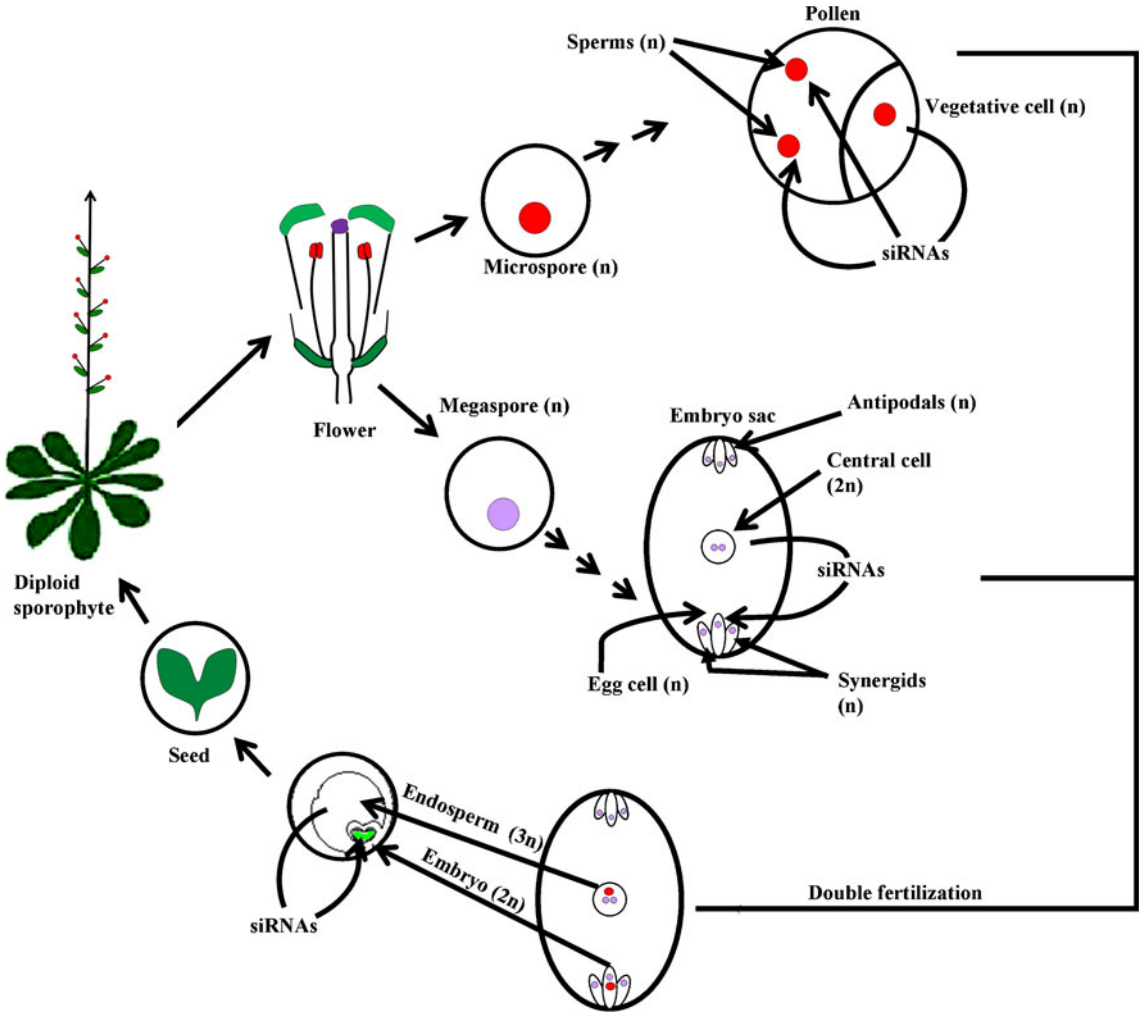

Figure 7. Schematic diagramme of the modulations in the genomic cytosine methylation pattern during gametogenesis and embryogenesis in plants; imprinting in endosperm and resetting of cytosine methylation marks in embryo. Flowers are produced on adult sporophyte (2*n*) late in life cycle, after vegetative growth has been accomplished in plant formed from embryo (2*n*) in the germinated seed. Meiosis in anthers produce microspores and in ovules, it produces megaspores. A microspore (*n*) undergoes a round of mitosis to produce a generative cell (*n*) and a vegetative cell (*n*). Another round of mitosis in the generative cell produces two sperm nuclei, both haploid. A megaspore (*n*) undergoes three mitotic divisions to produce seven cells in the embryo sac: a binucleate central cell (2*n*), an egg cell (*n*), two synergids (*n*) and three antipodals (*n*). The genome of vegetative cell in the microspore (pollen grain) undergoes ROS1, DME, DML2 and DML3 caused active demethylation leading to the activation of RdDM whereby siRNAs are synthesized. A population of siRNAs is shuttled from the vegetative cell to sperm nuclei where these are used to reinforce/reprogramme the epigenetic pattern. Correspondingly, RdDM is activated in the central cell wherefrom siRNAs are shuttled into the egg cell for the reprogramming of the female gamete. Following double fertilization, the fertilized central cell develops into endosperm (3*n*) and fertilized egg cell grows into an embryo (2*n*). There is passive and active loss of cytosine methylation in endosperm. Some genes are expressed from the central cell nuclei (female nuclei) and others from the male nuclei, in a process called imprinting. RdDM is activated. siRNAs are transferred from endosperm to embryo for resetting of the genotype × environment specific cytosine methylation pattern to be produced. Once established, the DNA methylation pattern in the embryo is maintained autonomously through many mitotic cell divisions. Both the vegetative cell in pollen grain and endosperm in embryo sac are terminal tissues (endosperm is lost in the dicotylednous plant such as *A. thaliana* but persists in monocotylednous plants such as wheat, rice and maize) and their significant function is to supply populations of siRNA respectively to sperm nuclei and embryo for gametes and embryo to reinforce their desirable cytosine methylation pattern.

cells–vegetative cell and gametes in pollen, central cell and egg cell in embryo sac–endosperm, antipodals and synergids–early embryo–late embryo/sporophyte (Jullien *et al.* 2012).

The genetical term imprinting implies preferential expression of genetic information from one of the two identical alleles, either from the allele of maternal origin or from the allele of paternal origin (Vielle-Calzada *et al.* 1999; Kinoshita *et al.* 2004). Imprinting is conserved in angiosperms. More than 300 genes have been found in *A. thaliana, Z. mays* and *O. sativa* that have differential parent-of-origin-wise expression. Imprinted genes whose maternal alleles are expressed as well as those whose paternal

alleles are expressed have been identified and characterized (table 6). The differential expression of maternal alleles occurs in central cell/endosperm and that of paternal alleles in endosperm. Imprinted genes remain silent in sporophyte and are expressed in endosperm (Jullien and Berger 2008, 2010; Chuck and O'Connor 2010; Bauer and Fischer 2011; Ikeda 2012; Jiang and Kohler 2012; Xiao 2012). They are rarely expressed in embryo. Only one gene of *Z. mays*, namely *MATERNALLY EXPRESSED IN EMBRYO 1* (*MEE1*), is known which is imprinted in endosperm and also in very early embryo (Jahnke and Scholten 2009). The significance of imprinting requires to be understood. Also, the common differential structure or specialized genomic locations of imprinted genes remain to be revealed.

Cytosine methylation marks are extensively removed in vegetative cell of pollen grain, central cell of embryo sac and in endosperm, via passive and active demethylation. siRNAs synthesized in vegetative cell of pollen, central cell of embryo sac and endosperm are transferred respectively to immediate precursor of sperm cells or egg cell and embryo. In the recipients of siRNAs, the RdDM pathway is used to reinforce the methylation mechanism such that genomic stability is achieved by the silencing of TE and genes are programmed for their progressive roles in sporophyte development (Slotkin *et al.* 2009; Berger and Twell 2011; Gutierrez-Marcos and Dickinson 2012; Ikeda 2012). Final resetting/correction of methylation marks occur in the embryo (Calarco *et al.* 2012; Ibarra *et al.* 2012).

Promotion of apomeiosis in RdDM pathway mutants

Functional female gametes arising from meiosis with unreduced (2*n*) chromosomal complement (apomeiosis) produce asexual seeds in apomictic species (Koltunow and Grossniklaus 2003). In *Z. mays*, mutants in the orthologs of

Table 6. A partial list of the genes imprinted in *A. thaliana.*

Gene	Origin of the expressed allele[a]	Activity/function of the gene product	Reference(s)
MEDEA (MEA)	M	Polycomb group complex SET-domain protein	Kinoshita *et al.* (1999); Vielle-Calzada *et al.* (1999)
FLOWERING WAGENINGEN (FWA)	M	Class IV HD-ZIP protein	Kinoshita *et al.* (2004)
FERTILIZATION INDEPENDENT SEED2 (FIS2)	M	Zinc-finger transcription factor	Tiwari *et al.* (2008)
MATERNALLY EXPRESSED PAB C-TERMINAL (MPC)	M	C-terminal domain of poly(A) binding protein	Vielle-Calzada *et al.* (1999)
ARABIDOPSIS FORMIN HOMOLOG 5 (FH5)	M	Actin regulator	Fitz Gerald *et al.* (2009)
HOMEODOMAIN GLABROUS 8 (HDG8)	M	Class IV HD-ZIP transcription factor	Gehring *et al.* (2009)
HDG 9	M	As above	As above
JUMONJI C DOMAIN 15 (JMJ15)	M	Histone Lys demethylation protein	Gehring *et al.* (2011); Hsieh *et al.* (2011)
Skp-1-Like 8 (ASK8)	M	Ubiquitin degradation pathway protein	Hsieh *et al.* (2011)
ASK10	M	As above	As above
SUPPRESSOR of DRM1, DRM2, CMT3 (SDC)	M	F-box protein, a part E3-ubiquitin ligase complex	As above
HDG3	P	Class IV HD-ZIP transcription factor	Gehring *et al.* (2009)
PHERES 1 (PHE1)[b]	P	MADS-box transcription factor	Kohler *et al.* (2005)
VARIATION IN METHYLATION 5 (VIM5)[c]	P	5-methylcytosine binding protein; a part of ubiquitin 3 ligase	Gehring *et al.* (2011); Hsieh *et al.* (2011); Luo *et al.* (2011); Zhang *et al.* (2011)
VIM1	P	As above	As above
VIM6	P	As above	As above
SU(VAR)3-9 HISTONE METHYLTRANSFERASE HOMOLOG 7 (SUVH7)	P	Histone methyltransferase	Gehring *et al.* (2011); Hsieh *et al.* (2011)
SUVH9	P	As above	As above
RNA POLYMERASE IVa (POL IVa)	P	DNA dependent RNA polymerase	As above
YUCCA 10 (YUC10)	P	Flavin monooxygenase	Gehring *et al.* (2011); Hsieh *et al.* (2011); Luo *et al.* (2011); Zhang *et al.* (2011)

[a]M, maternal; P, paternal; [b]also in *Oryza sativa*; [c]also in *O. sativa* and *Z. mays*.

Table 7. Relationships between biotic or abiotic stress, changes in epigenetic marks and adaptive value of changes in plants.

Nature of stress	Plant species	Changes in epigenetic marks	Effect(s) on gene regulation observed	Reference(s)
Salt/salinity	*Glycine max*	Decreased DNA methylation and increased H3K9me3 and H3K9ac in promoters and gene bodies of many genes	Induction of many transcription factors was observed	Song *et al.* (2012)
As above	*Oryza sativa*	Hypomethylation at transposons and genes involved in stress response	Genomewide alteration in gene expression profile	Karan *et al.* (2012)
Drought	As above	Many sites were affected in both drought tolerant and sensitive genotypes; demethylation: methylation :: >4:1 in leaves and roots at tillering stage under drought conditions in genotype-cum-tissue specific manner	Irrigation reversed the DNA methylation changes at 70% of the sites	Wang *et al.* (2011)
As above	*Triticum aestivum* and *Sorghum bicolor*	Genomewide hypomethylation	NA	Zhong *et al.* (2009); Agboola *et al.* (2012)
Salt, Aluminium, cold or oxidative stress	*Nicotiana tabacum*	Hypomethylation of genomic DNA	Up-regulation of stress response genes, including the *GPDL* gene for glycerophosphodiesterase protein	Wada *et al.* (2004); Choi and Sano (2007)
Salt and drought	*Mesenbryanthemum crystallinum*	Hypermethylation at CHGs	Switch from C3 to CAM photosynthesis	Dyachenko *et al.* (2006)
Drought	*Arabidopsis thaliana*	Enrichment of H3K9me3 and H3K4ac in promoter regions and of H3K23ac and H3K27ac in coding regions	Induction of stress responsive genes, including *R29A*, *R29B*, *RD20* and *AtGOLS2*	Kim *et al.* (2008, 2012)
As above	*Pisum sativum*	Hypomethylation of specific genes	Upregulation of expression of specific genes	Labra *et al.* (2002)
Drought or high temperature	*Arabidopsis thaliana*	Hypomethylation; specifically at the *CHR12* locus	Arrest of growth at shoot apical meristem	Mlynarova *et al.* (2007)
High temperature	As above	Demethylation and loss of H3K9me2, excepting at the *COPIA78* retrotransposon family sites	Up- and downregulation of expression in transposons	Tittel-Elmer *et al.* (2010)
Pseudomonas syringae DC3000	As above	Extensive hypomethylation of heterochromatin	Massive cell death	Pavet *et al.* (2006)
Cucumber mosaic virus	As above	The 2b protein of virus sequesters siRNA	Suppression of host RdDM process	Duan *et al.* (2012)
Low temperature	*Zea mays*	Genomewide demethylation occurred in root tissues	Zm MI1 gene was switched on	Steward *et al.* (2002)
As above	As above	Loss of H2A.Z from nucleosomes	Actin related protein, a part of *SWI2/SNF2* required for loading H2A.Z histone variant or nucleosome not synthesized	March-Diaz *et al.* (2008); Kumar and Wigge (2010)
Low temperature	*Antirrhinum majus*	Decreased DNA methylation	Transposition of *Tam3* enhanced	Hashida *et al.* (2006)
Heavy metals	*Trifolium repens* and *Linum usitatissimum*	Hypomethylation, including that of specific genes	Upregulated expression of specific genes	Alina *et al.* (2004)
Ionizing radiation	*Pinus silvestris*	Genomic hypermethylation	NA	Kovalchuk *et al.* (2003)
Salt marsh	*Laguncularia racemosa*	Genome of population group near salt marsh was hypomethylated as compared to riverside population	NA	Lira-Medeiros *et al.* (2010)
Salt or alkali	*Gossypium hirsutum*	Hypomethylation	Tolerance to stress	Zhao *et al.* (2010); Cao *et al.* (2011)

NA, information not available.

Table 8. Evidence for the transgenerational (Lamarckian) inheritance of epigenetic marks in plants acquired upon exposure to abiotic or biotic stresses or hybridization with a parent carrying such marks (epiallele(s)) that provide protection to future generations.

Plant	Stress/hybridization shock received in parental generation	Features of transgenerationally inherited marks and related phenotype in progeny, different from those in the progeny of control plant	Reference(s)
Zea mays	*B*-1 × *B*′ hybidization	*B*-1 allele was paramutated to *B*′ and *B*′*B*′ status was transferred to next generation which was distinct from that in the normal *B*-1 *B*-1 plants	Reyes (2006); Kumar (2006)
Arabidopsis thaliana	Salinity stress	Progeny had hypermethylated gene promoters and exons, chromatin enriched with H3K9me and depleted of H3K9ac in histones and gene expression at low level in many stress responsive genes; *DRD2*, *DDL* and *AGO6* genes of siRNA biogenesis were also hypermethylated	Billichak *et al.* (2012)
As above	*Pseudomonas syringae* pv tomato DC 3000	Progeny plants were hypomethylated, like *drm1 drm2 cmt3, ddm1* or *met1* plants, and were tolerant to PsDC3000 infection	Katiyar-Agarwal *et al.* (2006); Luna *et al.* (2012); Dowen *et al.* (2012)
As above	Priming of infection by use of Pst avr Rpt2 avirulent derivative of PsDC300 or β-amino butyric acid	Protection against the virulent *Pseudomonas syringae* and downy mildew pathogen *Hyaloperonospora arabidopsidis* for several generations; further priming improved pathogen tolerance	Slaughter *et al.* (2012)
As above	Heat	Progeny demonstrated the phenotype of *arp6* mutant by constitutively expressing actin related protein, a part of *SWI2/SNF2* nucleosome assembly required for loading H2A.Z histones into promoters	Kumar and Wigge (2010)
As above and *Solanum lycopersicon*	Insect herbivory	Toleration towards pests and pathogens lasted for two generations. RdDM function was required for heritable resistance to herbivory	Rasmann *et al.* (2012)
Arabidopsis thaliana	Exposure to UV-C radiation, high or low temperatures or *Peronospora parasitica* infection	Hypomethylation related enhanced frequency of inheritable somatic and/or meiotic recombination was inherited	Lucht *et al.* (2002); Molinier *et al.* (2006); Boyko *et al.* (2007); Yao and Kovalchuk (2011)
As above	Jasmonic acid and salicylic acid treatment	Toleration against herbivores	Latzel *et al.* (2012)
As above	Cytosine methylase deficient genetic background	High frequency recombination in hypomethylated chromosome arms was noted	Mirouze *et al.* (2012)
As above	Heat	In the progeny of heat-stressed *pol IV, pol V* and *rdr2* mutants transposition of *ONSEN* transposable elements was detected but not in the generation of treatment	Ito *et al.* (2011); Matsunaga *et al.* (2012)
As above	5-azacytidine treatment of plants stressed by salt, cold, heat and UVC	Global hypomethylation accompanied by increased stress tolerance and higher levels of recombination	Boyko *et al.* (2010)

Table 8. (*contd.*)

Plant	Stress/hybridization shock received in parental generation	Features of transgenerationally inherited marks and related phenotype in progeny, different from those in the progeny of control plant	Reference(s)
As above	Potato spindle tuber viroid (PSTVd)	F_0 PSTVd transgenic plants infected with PSTVd were defended by densely *de novo* methylated CG, CHG and CHH sites. The acquired defence in progeny plants was predominant due to CG methylations in promoters and gene bodies	Dalakouras *et al.* (2012)
As above	Pathogen(s), benzo(1,2,3)thia-diazole-7-carbo-thioic acid 5-methyl ester or salicylic acid	Induction of *HsfB1* allows tolerance to heat, systemic acquired resistance to pathogens and also to abiotic stresses	Pick *et al.* (2012)
Nicotiana tabacum	TMV infection	Elevated levels of homologous recombination associated with global genome methylation, specific hypomethylation at loci associated with TMV resistance and general stress response and general tolerance towards common pathogens	Wada *et al.* (2004); Boyko *et al.* (2007); Kathiria *et al.* (2010)
Oryza sativa	*Xanthomonas oryzae* pv *oryzae* race PR2	Progeny of tolerant plant, due to aza-deoxycytidine caused hypomethylation, continued to be tolerant	Akimoto *et al.* (2007)
As above	5-aza deoxycytidine treatment	Treated plants and progeny continued to be hypomethylated and dwarf in habit	Sano *et al.* (1990)
As above	N-deficiency stress	Half of the locus specific methylation changes and associated tolerance towards N-deficiency were inherited through two generations	Kou *et al.* (2011)
As above	Treatment of seedlings with 50 μM $CdCl_2$, $CrCl_3$, $CuSO_4$ or $HgCl_2$	Hypomethylation accompanied by tolerance to the heavy metal at 500 μM concentration	Ou *et al.* (2012)
Linaria vulgaris	Spontaneous (?)	Hypermethylation led silencing of *CYCLOIDEA* gene leading to change in flower structure has lasted many generations	Cubas *et al.* (1999)
Solanum lycopersicon	As above	Hypermethylation led evergreen fruit character has lasted many generations	Schmitz *et al.* (2011)
Taraxacum officinale	Herbivory, jasmonic acid, salicylic acid, salt stress and low nutrient treatments	Toleration against pests and pathogens and hypomethylation were inherited	Verhoeven *et al.* (2010)
Dactylorhiza majlis, *D. traunsteineri* and *D. ebudensis*	Alloploidization	Adaptation to new geographical and ecological niches	Paun *et al.* (2010)

CMT3, *DRM2*, *RDR2* and *AGO4/6* genes have been found to produce multiple asexual embryo sacs/embryos/seeds (Garcia-Aguilar *et al.* 2010; Olmedo-Monfil *et al.* 2010; Singh *et al.* 2011). These observations suggest that regulation of DNA methylation in precursor cells of female gametes plays a critical role in gametogenic differentiation such that unreduced embryo sac(s), central cell and/or egg cell arise. Partial sterility observed in DNA methylation mutants of *A. thaliana* may be related to low rate occurrence of apomeiosis in their flowers.

Stress induced DNA methylation changes and associated defence to stress and heritability of the acquired changes

Being sessile, plants get exposed to many kinds of stress conditions of physical, chemical or biological origin; among them salinity, drought, herbivory and pathogen attack are particularly important in agriculture. In nature, plants may get exposed to similar stresses over several contiguous generations. Plants respond to a stress by synthesizing certain metabolites, proteins and small RNAs that may provide them protection/immunity against a particular stress or some other stresses as well. Tables 7 and 8 exemplify adaptive/acquired defence against stress in several plant species. In some examples, the acquired defense against stress was transgenerational (table 8). In all cases, the acquired defence was found correlated with the changes in the cytosine methylation pattern in the genomes of plants exposed to stress. These changes represented memory of the stress and physiological response to stress. A mechanism of acquirement of defence in *A. thaliana* against *Pseudomonas syringae* is diagrammed in figure 8. Hypomethylation was a common feature found associated with adaptive defence against various stresses. *A. thaliana* plants inoculated with *P. syringae* pv tomato DC 3000 became tolerant to the pathogen and simultaneously their genome became cytosine demethylated (Dowen *et al.* 2012; Luna *et al.* 2012; Slaughter *et al.* 2012). The pathogen resistant phenotype of single *ddm1* and *met1* mutants and triple mutant *drm1*, *drm2* and *cmt3* altered tolerance in *A. thaliana* against the bacterial infection was because of demethylation in DNA. Further, the progeny of F_0 *A. thaliana* plants primed by repetitive inoculation with virulent or avirulent *P. syringae* or treated with β-aminobutyric acid, developed tolerance against virulent *P. syringae* as well as *Hyaloperonospora arabidopsidis*. Both F_0 and F_1 plants had enhanced expression of *PATHOGENESIS RELATED 1, WRKY6* and *WRKY53* genes and also possessed the property of DNA demethylation. This demonstrated that acquired defence via demethylation in DNA is heritable. Since in some experiments summarized in table 7, acquired defence against stress was not transgenerational, an explanation about the discrepancy is required. As shown in figure 9, the transgenerational inheritance may not be seen where transfer of epigenetic changes to embryo did not occur in gametes.

The evidence suggests that the acquired/adaptive tolerance to stress in plant organs results from the stress induced acquirement of desirable epigenetic marks in their cells

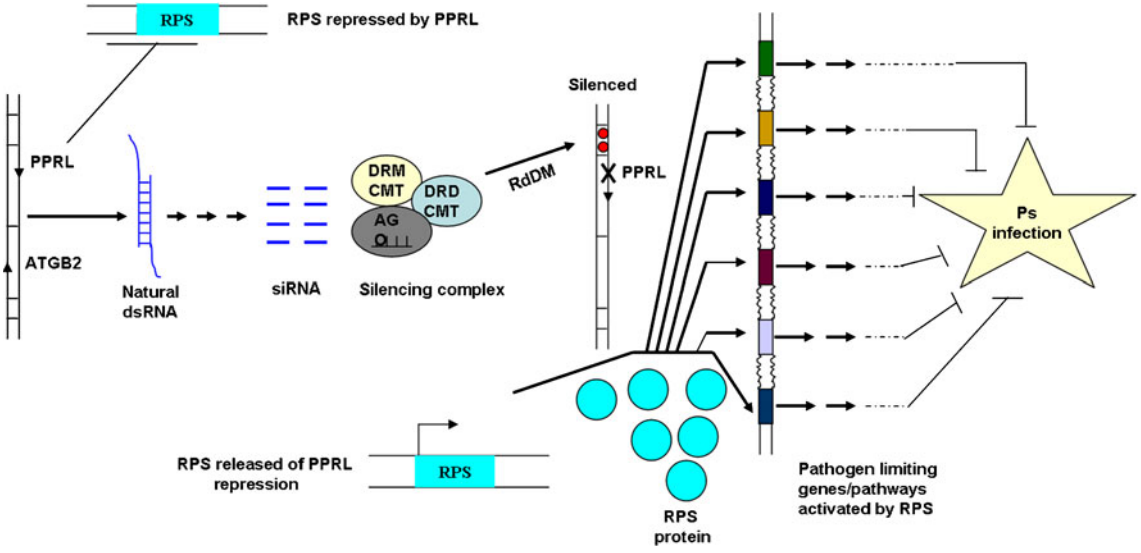

Figure 8. Scheme for the provision of immunity to *Pseudomonas syringae* (*Ps*) infection in *A. thaliana* by the action of siRNA synthesized by the mediation of RDR and DCL orthologues. The transcription events from *PPRL* and *ATGB2* genes produce sense–antisense dsRNA for a common region. This naturally formed dsRNA is processed into siRNA by the intermediation of RDR and DCL3. The siRNAs loaded on AGO4 are used by the signalling machinery to target methylation on PPRL sequences. The silencing of PPRL releases the R gene RPS from PPRL imposed repression. RPS then activates genetic pathways that variously control the *Ps* infection.

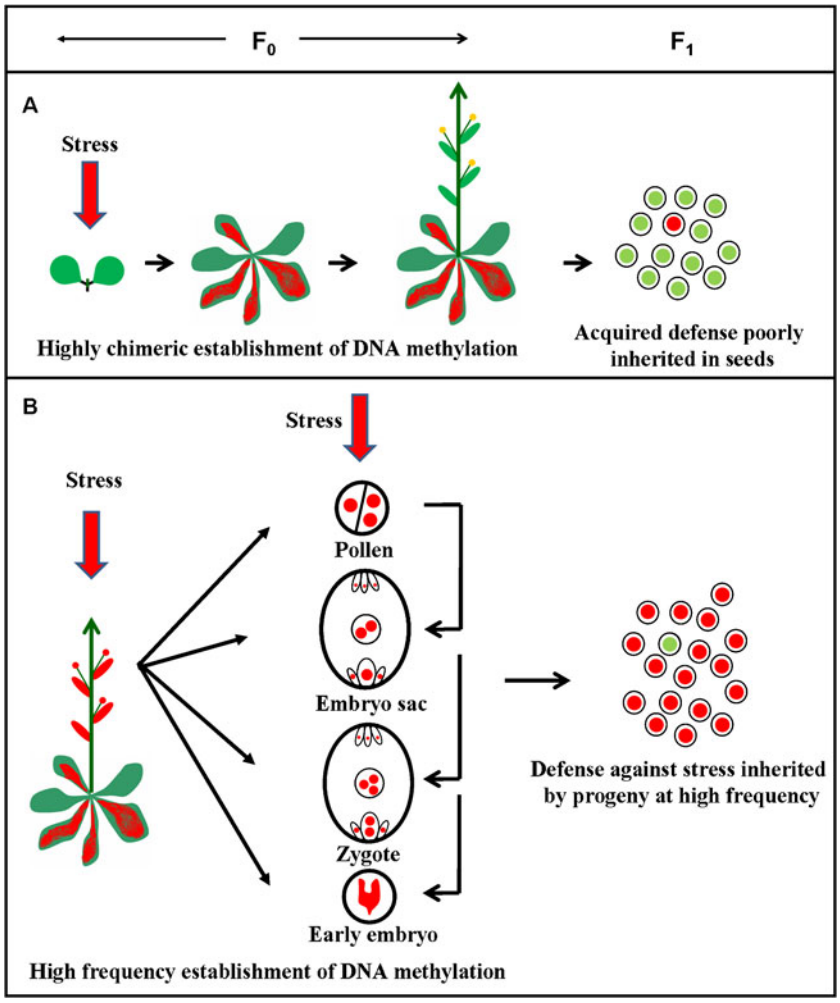

Figure 9. Diagramme showing conditions of exposure to stress and degree of heritability of acquired defence against stress in plants. (A), a seedling is shown to be exposed to stress such as heat, β-aminobutyric acid, salicylic acid or jasmonic acid. The adult plant obtained from the stressed seedling is a stress tolerant chimera, because DNA methylation modifications that occurred in meristematic stem cells got inherited mitotically only by some of the leaf and flower primordia. Only a few seeds inherited the stress adaptive methylation marks from such a plant. (B), when a plant received the stress in the course of its vegetative and reproductive development, most of its cells/tissue/organs, especially gametophytes in flowers inherited mitotically and meiotically the stress adaptive DNA marks. The adapted character will persist in lineage until the adaptive methylation marks are changed. Red colour indicates establishment of stress induced DNA methylation.

(tables 7 and 8). Epigenetic changes must occur in stem cells so that the shoot organs and root system develop stress tolerance. If seedlings or juvenile plants get exposed to stress, the vegetative organs may demonstrate stress adaptation but the progeny may not. Because of the depletion of epigenetically modified stem cells, epigenetic changes may not be passed through gametes to progeny embryos (figure 9). The flowers, microspores, pollen grains, megaspores, embryo sacs, endosperms or nascent embryos borne on F_0 plants must either receive the stress induced epigenetic marks from their parental stem cells or must themselves undergo the stress induced desirable epigenetic modifications, for the transgenerational inheritance of stress adaptation to be observed in the F_1 generation (figure 9).

DNA methylation pattern changes during development

Above, evidence in several plant species (tables 7 and 8) was presented which shows that exposure to abiotic and biotic stress produced changes in overall DNA methylation such that plants acquired tolerance towards the concerned stress(es). These observations suggested that defence against the varied stresses must be a consequence of gene expression changes in plant organs that altered the plant physiology such that plants became stress tolerant. There is more direct evidence that correlates cell, tissue and organ differentiation in seed, root, leaf and flower with specific changes in DNA methylation patterns. DNA methylation differences

Table 9. Experimental evidences for the roles of epigenetic changes in plant development.

Plant species	Role	Reference(s)
Arabidopsis thaliana and *Nicotiana tabacum*	Leaf, inflorescence and flower differentiation and flowering time determination are compromised in *met1* and *ddm* mutants; above and reduced growth are observed in *drm1 drm2 cmt3* mutants; flowering time is delayed in *pol IV* and *pol V* mutants; loss of freezing tolerance and delay in flowering and longer life cycle occur in *HDA6* mutant axe 1-5; high degree of transposon movement is observed in *cmt3 met1* mutants, and *dme, ros, dml1* and *dml2* mutants show some seed abortion. Therefore, the roles include regulation of gene network for cytosine methylation and maintenance of genome stability and organ development and seed fertility	Finnegan *et al.* (1996); Nakano *et al.* (2000); Kato *et al.* (2003); Vanyushin (2006); Chan *et al.* (2006a, b); Wu *et al.* (2008); Ream *et al.* (2009); To *et al.* (2011a, b)
Arabidopsis thaliana, *Zea mays* and *Oryza sativa*	Gene imprinting or selective expression of individual genes of maternal or paternal origin in endosperm; more than 300 genes have demonstrated differential parent-of-origin-wise expression in endosperm	Kohler *et al.* (2005); Bauer and Fischer (2011); Raissig *et al.* (2011); Ikeda (2012); Xiao (2012)
Zea mays	Mutants of *CMT3*, *DRM2* and *AGO* homologues formed unreduced gametes and multiple embryo sacs/ovules or apomixis like phenotype	Garcia-Aguilar *et al.* (2010); Singh *et al.* (2011)
Gossypium	Regulation of gene networks for plant development associated with extensive DNA hypomethylation leading to heterotic vigour	Zhao *et al.* (2008); Singh *et al.* (2011)
Brassica rapa	Self incompatibility phenotype is determined by *de novo* DNA methylation in tapetum of SP11 promoter. At the S locus *SRK* gene and SP11 are closely linked and there are two class I and class II haplotypes, among them, class I is dominant. In class I/class II heterozygotes, siRNA synthesized from SP11 of class I target the SP11 promoter of class II SP11. Consequent hypermethylation leads to self incompatibility.	Shiba *et al.* (2006); Tarutani *et al.* (2010)
Arabidopsis thaliana	Heterochromatin silenced by HDA6 and MET1 interaction; ectopic expression of many euchromatic loci	To *et al.* (2011a, b)
As above	By measuring meiotic crossing over (CO) along length of chromosomes in isogenic epigenetic recombinant inbred lines a recombination map was constructed which showed that CO was higher in chromosome arms due to suppression of CO in heterochromatic pericentromeric regions, irrespective of the cytosine methylation status	Colome-Tatche *et al.* (2012)
As above	DNA methylation sequencing of recombinant inbred lines derived over 30 generations of selfing revealed that the epimutation rate was ten thousand times higher than the DNA sequence mutation rate	Ossowski *et al.* (2010); Schmitz *et al.* (2011)
Phoenix dactylifera	Branching related to demethylation in axillary buds	Fang and Chao (2007)
Solanum lycopersicon, *Arabidopsis thaliana*, *Oryza sativa* and *Zea mays*	Higher levels of DNA methylation in seeds and mature/senescent leaves than in nascent leaves and in stems and roots	Messeguer *et al.* (1991); Xiong *et al.* (1999); Ruiz-Garcia *et al.* (2005); Sha *et al.* (2005); Brown *et al.* (2008); Lu *et al.* (2008)
Arabidopsis thaliana	DNA methylation activity fluctuates during reproduction; maintenance DNA methylases are deficiently expressed in gametogenesis and strongly in postfertilization-nascent embryo	Jullien *et al.* (2012)
As above and *Beta vulgaris* cv Altissima	By study of *UBIQUITIN EXTENTION PROTEIN 6* (UBI6) gene in *in vitro* cultures undergoing root and shoot organogenesis, organ specific DNA methylation marks were observed which were conserved in derived plants	Maury *et al.* (2012)

Table 9. (*contd.*)

Plant species	Role	Reference(s)
Mimulus guttatus	Hypermethylation led downregulation of *MgMYBML8* inheritably increased density of trichomes	Scoville *et al.* (2011)
Populus	Horizontal transfer of *PtDDM-RNAi* transgene reduced DNA methylation and caused mottling in leaves	Zhu *et al.* (2013)
As above	Winter dormancy was associated with global hypermethylation	Conde *et al.* (2013)
Elaeis guineensis	Global hypomethylation is associated with mantled phenotype or homeotic changes of stamens into male flowers and staminoides into female flowers	Jaligot *et al.* (2004); Rival *et al.* (2008)

Table 10. Effect of DNA methylation deficiency on fertility (flower organ abnormalities) cum seed viability (embryonic abortion) in *A. thaliana.*

Mutated gene(s) in homozygous condition[a]	Phenotype	Reference(s)
met1	Marginal sterility[b]	Finnegan *et al.* (1996); Kankel *et al.* (2003)
ddm1	As above	Brzeski and Jerzmanowski (2003)
met1 ddm1	As above	Kakutani *et al.* (1996)
drm1 drm2	Normal	Cao and Jacobsen (2002a, b)
cmt3	As above	Lindroth *et al.* (2001)
met1 cmt3	Marginal sterility	Xiao *et al.* (2006)
drm2 cmt3	Partial sterility[c]	Cao and Jacobsen (2002a, b); Cao *et al.* (2003)
drm1 drm2 cmt3	As above	Cao and Jacobsen (2002a, b); Henderson and Jacobsen (2008)
rdr2	As above	Law *et al.* (2011)
dcl3	As above	Henderson *et al.* (2006)
ago4	As above	Zilberman *et al.* (2003); Qi *et al.* (2006); Henderson and Jacobsen (2007)
drd1	As above	Kanno *et al.* (2004); Law *et al.* (2010)
drd1 cmt3	As above	Chan *et al.* (2006a, b); Law *et al.* (2010)
dms4	As above	Kanno *et al.* (2010); Law *et al.* (2010); Greenberg *et al.* (2011)
Kyp	Normal	Jackson *et al.* (2002)
drm1 drm2 kyp	Partial sterility	Xiao *et al.* (2006); Chan *et al.* (2006a, b)
nrpd1a nrpd2a (pol IV)	Normal[d]	Ream *et al.* (2009); Huang *et al.* (2009); Law *et al.* (2010)
nrpd1b nrpd2b nrpe5-1 (pol V)	As above	As above
nrpd2a nrpd2b (pol II) cmt3	Partial sterility	Xiao *et al.* (2006); Law *et al.* (2011)
nrpd2a nrpd2b drm1 drm2	Normal	As above
drd1 drm1 drm2	As above	As above
Dme	Partial sterility	Choi *et al.* (2002); Jullien *et al.* (2006); Xiao *et al.* (2006); Penterman *et al.* (2007); Zhu *et al.* (2007); Hsieh *et al.* (2009); Gehring *et al.* (2009); Wu and Zhang (2010)
dml1 dml2	As above	As above
ros1	As above	Gong *et al.* (2002)
had	Normal	Probst *et al.* (2004); Zilberman *et al.* (2008)
Mbd	Partial sterility[e]	Ng *et al.* (2000); Scebba *et al.* (2003); Zemach and Grafi (2003, 2007); Zemach *et al.* (2005)

[a]*met1 = methyl transferase1*; *ddm1 = deficient in DNA methylation1*; *drm1, 2 = domain rearranged methyl transferase*; *cmt3 = chromomethylase3*; *rdr2 = RNA dependent RNA polymerase 2*; *dcl3 = dicer3*; *ago4 = argonaute4*; *drd1 = defective in RNA directed DNA methylation1*; *dms4 = defective in meristem silencing4*; *kyp = kryptonite*; *nrpd1a, 2a = nuclear RNA polymerase DNA dependent*; *pol IV*; *pol V*; *pol II*; *hda = histone deacetylase*; *mbd = methyl-Cp-binding domain protein*.
[b]Reduced height, more branching, changes in flower organ identities and numbers, delayed flowering.
[c]Curled leaves, retarded plant growth, reduction in biomass.
[d]More rosette leaves, delayed flowering.
[e]Late flowering.

have been reported between nascent organs and nascent versus senescent organs (table 9, rows 10–12). Hypomethylation in axillary meristems in *Phoenix dactylifera* was reported to be associated with the development of branches (table 9, row 9). In cotton (table 9, row 4) genomewide hypomethylation favoured higher levels of growth in the development of organs such that hybrid vigour was manifested in cotton fiber yield. In poplar winter dormancy and vegetative growth in spring are respectively associated with global hypermethylation and hypomethylation (table 9, row 15). The above examples suggest that changes in DNA methylation patterns modify the functioning of regulatory gene networks.

Impairment of reproductive potential by defects in DNA methylation maintenance and establishment pathways

Large phenotypic differences have been observed between the mutant plants deficient in establishing and/or maintaining cytosine methylation marks and corresponding wild-type plants. The pleiotropic phenotype of *met1* plants includes curled leaves, reduced apical dominance, late flowering, floral abnormalities and poor seed setting. The *ddm1* mutants are reported to flower late and carry flowers and leaves of altered morphologies. Triple *drm1 drm2 cmt3* mutants produce lesser biomass and seeds. The *pol IV* and *pol V* form more of rosette leaves and flower late. The phenotypes of above mutants and of others affected in DNA methylation process are summarized in table 9, rows 1–3 and table 10. Altogether, the evidence shows that methylation deficiency adversely affects plant's reproductive system.

Mutagenicity at methylated cytosine sequences

5-Methyl cytosine is an endogenous mutagen in DNA; the sites of DNA methylation are mutational hotspots (Pfeifer 2006; Walsh and Xu 2006). Cytosine methylation (^{m}C–G) sites are known to undergo mutation at 35–150 times higher rate than sites occupied by C–G and A–T base pairs. Spontaneous random deamination of methylated cytosines in DNA result in C→T or G→A transitions. Sunlight induces pyrimidine dimers in sequences where methylated cytosine is located adjacent to another pyrimidine. The repair of such events results in G→T transversions. All conditions that lead to active demethylation of DNA, such as exposure to abiotic and biotic stresses and interspecies hybridizations (amphiploidy) create opportunities for massive mutagenesis at cytosine methylation sites (Zhu 2009). The DNA glycosylases ROS1, DME, DML2 and DML3 led active DNA demethylation in plants is highly mutagenic because they

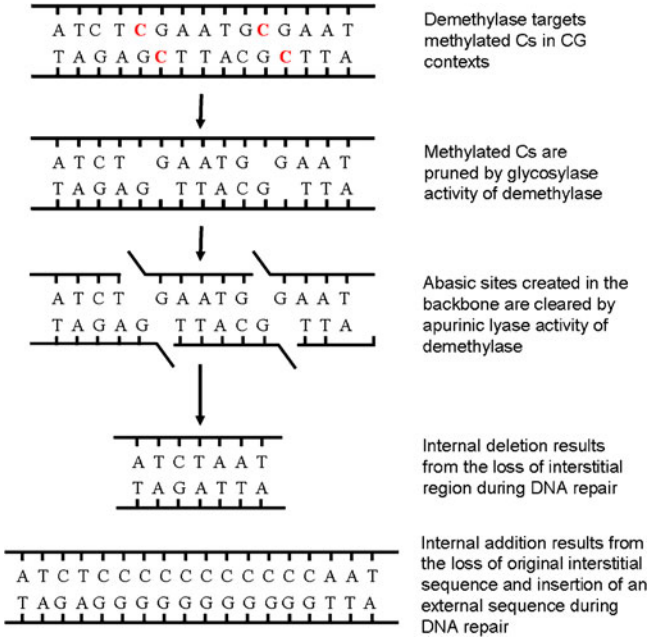

Figure 10. Scheme showing induction of deletion and addition mutations consequential to removal of methylated cytosines at nearby locations on opposite DNA strands. Methyl cytosines are a kind of endogenous mutagen and DNA sequences containing them are mutational hotspots.

create a nick in the backbone of DNA strand at the site of their action. Following hydrolysis of the glycosidic bond between deoxyribose and the methylated cytosine base, their apurinic/apyrimidic lyase activity causes a nick such that a gap is created in the DNA strand (figure 6). This gap is a potential site for a frameshift, transition and transversion mutation. If nicks get created in both the strands at a ^{m}C–G::G–^{m}C site in DNA, the result will be a double strand break. This site will be eligible for repair leading to a variety of mutations, including an insertion. Simultaneous occurrence of two such events can result in loss of intervening DNA causing a deletion mutation. Such a site could also result in an addition mutation (figure 10). Somaclonal variation (Jaligot *et al.* 2004; Rival *et al.* 2008; Bairu *et al.* 2011) is attributable to mutations at cytosine methylation sites. It appears that plant genomes are tailor made for generation of new genetic variation due to innate mutagenic properties of methylated cytosine sites in DNA.

Spontaneous origin of epialleles carrying DNA methylation marks

Previous section concerned burst of epigenetic variation following exposure of plants to abiotic and biotic stresses. It also suggested requirements for the stress induced epigenetic

changes for being heritable. Here the spontaneous origin of epigenetic changes without the intervention of environmental perturbations is discussed.

In the *A. thaliana* nuclear genome about 70% of cytosines in CG context are methylated; cytosines in CA, CT and CC arrangements are also methylated in some measure (Maunakea *et al.* 2010; Brenet *et al.* 2011). The cytosine methylations give rise to locus-wise patterns of DNA methylation polymorphism. An epiallele of a locus differs from other epialleles of that isogenic locus in its differential cytosine methylation pattern. The patterns of cytosine methylation at the various loci of genome are inherited from cell to cell with the mediation of maintenance methyltransferases. Epialleles result from the gain or loss of methylation at cytosine sites at a locus that are methylation sensitive. The spontaneous variation in methylation patterns arises on account of errors in epigenomic replication. Whereas the genomic replication process is robust, the maintenance methyltransferases are somewhat inefficient in copying the parental epigenetic marks in mitotic cycles. Consequently, epigenetic polymorphism arises (Vaughn *et al.* 2007). Epialleles at a locus can have distinct phenotypes. Plants differing in just one epiallele can be phenotypically different. Thus, formation of epialleles for various loci in genome increases phenotypic variability in plants.

Two types of long-term experiments have provided critical information about the spontaneous rate of induction of DNA methylation polymorphism and properties of epigenetic polymorphism prevailing in plants. In one kind of experiments, variation in DNA methylation pattern was compared between parents widely differing in cytosine methylation marks and their progenies derived by selfing and single seed descent over 30 generations in a constant environment (Becker *et al.* 2011; Schmitz *et al.* 2011). In the second type of experiment, the *met1* and *ddm1* mutants were respectively crossed with isogenic *MET1* line and *DDM1* line and the epigenetic recombinant inbred lines (epiRILs) were constructed and characterized from cytosine methylation pattern polymorphism and expression of quantitative traits, in constant genetic background. In the *met1* × *MET1* cross, in an early generation only *MET1* bearing RILs were carried forward (Johannes *et al.* 2009; Reinders *et al.* 2009; Teixeira *et al.* 2009; Sasaki *et al.* 2012a, b). The first kind of experiment revealed that, whereas the spontaneous rate of base substitutions per site per generation, at the DNA sequence level, was 7×10^{-9}, the rate of spontaneous induction of methylation polymorphism per CG per generation was 4.46×10^{-4}. Together the two experiments revealed the following about cytosine methylation related epigenetic variation. (i) *met1* mutation causes nearly complete erasing of methylation marks at CG sites. In the presence of *MET1* allele, restoration occurred in region specific manner over many generations and was positively influenced by transcription. Methylation of a single CG could silence a gene. *met1* kind of mutation arose spontaneously once in 30 generations. (ii) There are hotspots for development of methylation marks. Sites located outside of nucleosomes, sites adjacent to noncoding DNA, promoters, enhancers, exons adjacent to promoters are readily methylated. DNA methylation occurs in all the three contexts (CG, CHG and CHH). CG methylation is present in gene bodies and is enriched towards 3′ end. The introns are twice more methylated than exons. Heterochromatin is rich in all the three marks. (iii) Epigenetic changes can originate spontaneously over many loci rapidly; however, they are reversible. (iv) Preexisting patterns are often lost. Certain lost patterns have low chance of reformation. (v) When parents have very divergent epigenetic patterns, the stability of existing patterns is low and novel patterns keep developing. Majority of epialleles are stable across many generations.

There are several epigenetic mutants that have persistent epigenetic marks (hypermethylation) which arose in nature and got selected on account of their stability (table 8, rows 19, 20 and 22). In *Linaria vulgaris*, the epimutation prevents expression of a *Cycloidea*-like gene and thereby mutant plants have acquired radial symmetry in flowers (Cubas *et al.* 1999). In *Solanum lycopersicon*, the epimutation has silenced the *SPL-CNR* gene which encodes a *SBP-box* (*squamosa promoter binding protein-like*) transcription factor imparting nonripening (evergreen) phenotype to fruits (Manning *et al.* 2006). In rice D1 (*DWARF 1*) gene has been silenced to give *Epi-d1* dwarf epimutant (Miura *et al.* 2009).

Cytosine methylation is a highly versatile plant mechanism for rapid creation of enormous new variability from genetic potential already present in species.

Paramutation: direct copying of epiallele between homologous DNA sequences

Paramutation results from interaction between two alleles of a gene such that an allele is heritably changed by the other allele. It is well exemplified by the *booster-1* (*b*-1) locus in maize (*Zea mays*) (figure 11). The *b*-1 locus encodes the transcription factor bHLH which activates the genes of anthocyanin biosynthetic pathway. *B*-1 (paramutable, active allele) and *B′* (paramutagenic, relatively inactive allele), the two alleles at the *b*-1 locus have the same DNA sequence but differ in the pattern of DNA methylation. In the F1 of *B*-1 × *B′* cross, the *B′* allele silences the *B*-1 allele, resulting in the weakening of *bHLH* expression and acquirement of paramutagenicity by *B*-1 allele. Paramutation requires transcripts from bidirectional expression of tandem repeats located 100 kb upstream of the *bHLH* gene. Pol IV is involved in the transcription of tandem repeats. Transcripts are processed by *MOP* (a *RDR2* homolog) and *DCL* to generate siRNAs. The RdDM silencer complex then silences the *b*-1 locus of *B*-1 allele by DNA methylation. The F_1 plant now has both the alleles at *b*-1 locus paramutated via their DNA methylation by use of RdDM pathway (Stam *et al.* 2002; Alleman *et al.* 2006; Kumar 2006; Chandler 2007; Erhard *et al.* 2009; Stonaker *et al.* 2009). Paramutation appears to

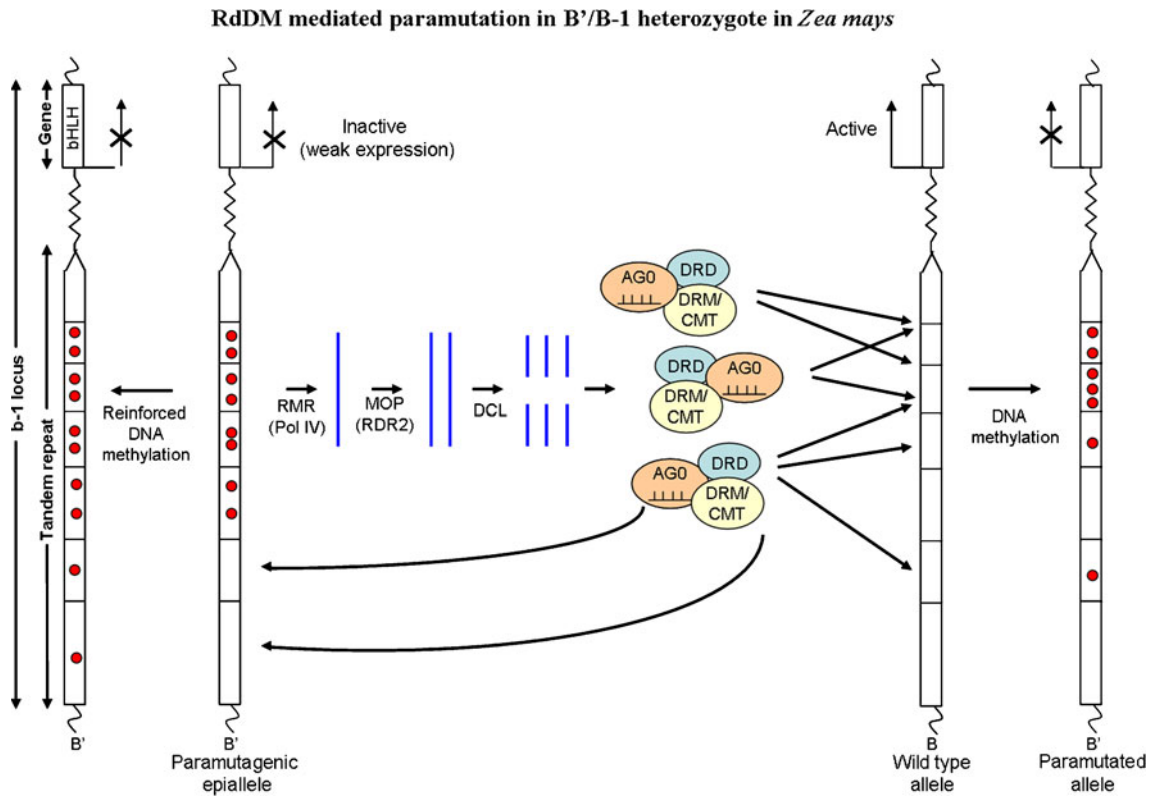

Figure 11. Scheme of paramutagenization of *B*-1 normal allele to *B′* paramutated epiallele in *Z. mays*. The enhancer element of the *B*-1 gene located 100 kb upstream has seven copies of a 853-bp sequence. The tandem repeats are transcribed bidirectionally by the combined action of DNA dependent RNA polymerase IV (RPD, RMR7). Upon their RNA dependent RNA polymerase (MOP) based conversion into double stranded RNA followed by DCL3 action, siRNAs complementary to the enhancer element are formed. The AGO four loaded with siRNAs possessing different specificities along 853 bp element together with DRM 2 or CMT 3 and components of the DDR complex (figure 4) target DNA sites and *B′* chromosomes and methylate them. In the process, while DNA methylaton is maintained on *B′* chromosome, the B-1 chomosome is also converted into *B′* state or paramutated. The diagramme given in figure 5 explains the individual roles of the components in DDR complex. The red dots are sites of DNA methylation.

be a property shared by genes that lie close to multiple repeated sequences/transposons. It is a mechanism by which DNA sequences in embryo contributed by a gamete rapidly acquire the epigenetic pattern already present in homologous sequences contributed by the other gamete. Paramutation homogenizes the epigenetic patterns on homologous sequences in diploids and polyploids.

A process of copying of epiallele already present at a locus at other copy(ies) of that locus, endogenous or acquired transgenically, analogous to paramutation has been observed at the *FWA* locus in *A. thaliana* (figure 12). FWA protein ectopically expressed in leaves interacts with FT protein, latter a mobile flowering signal expressed from the gene *FLOWERING LOCUS T (FT)*, and thereby flowering is delayed. Normally *FWA* is inactive in vegetative tissues because of hypermethylation at its promoter which carries two pairs of tandem repeats and the short interspersed nuclear element (SINE). The *FWA* promoter is methylated by the RdDM pathway via synthesis of siRNAs specific to the promoter. The transgenic *FWA* is silenced by the RdDM because of the availability of siRNAs for the promoter region, much like the paramutation process (Kinoshita *et al.* 2007).

Regulation of meiotic recombination by DNA methylation marks

The genetic variability in a plant population is amplified by genetic recombination. In anthers and ovules of plant flowers, crossing over between homologous chromosomes at meiotic cell divisions leads to reciprocal genetic exchange (or genetic recombination). Crossing over at meiosis is a genetically determined trait and meiotic recombination rate varies between species. It is well known that crossing over events occur at higher frequency in chromosome arms as compared to near the centromere, the chromosome region which has abundance of repeat sequences. There is thus inverse relationship between meiotic recombination rate (MRR) and DNA methylation intensity. This relationship has been investigated in wild type × wild type, *MET1* (wild

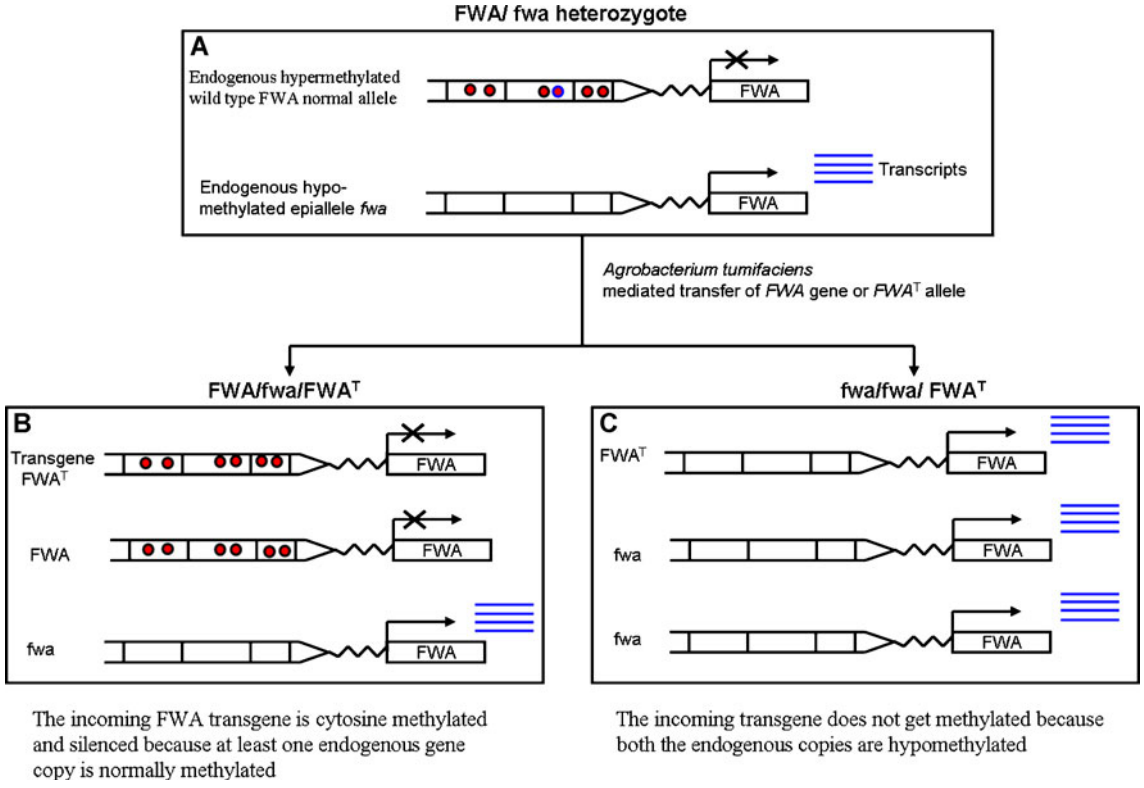

Figure 12. Determination of the cytosine methylation at the promoter of *FWA* transgene (FWA^T) by the cytosine methylation state in the endogenous copies of *FWA* gene. In (A), the diploid *A. thaliana* cell is a heterozygote; it has an *FWA* gene with wild-type promoter that gets hypermethylated and another copy of *FWA* gene with unmethylated/hypomethylated promoter called *fwa*. The *fwa* state is generated spontaneously in MET1 background and necessarily in *met1* background. Since the wild-type allele is silenced, *FWA* structural gene is expressed from the *fwa* epiallele. In (B), a cell is shown which has received wild-type *FWA* transgene. Here the FWA^T and *FWA* are methylated but *fwa* remains unmethylated. In (C), a *fwa/fwa* cell which has received FWA^T is shown. Here, all the three gene copies remain unmethylated. The presence of methylated *FWA* promoter is essential for the methylation of FWA^T transgene. However, *fwa* remains unmethylated even in the presence of a methylated *FWA* wild-type gene copy. The methylation of DNA follows the pathways depicted in figure 4 (modified from Kumar 2007).

type) × *met1* and *DDM1* (wild type) × *ddm1* crosses. It is recalled that in comparison to wild types, the *met1* and *ddm1* lines were 70% cytosine demethylated. It was observed that the demethylated *met1* and *ddm1* chromosomes stimulated recombination in hypomethylated chromosome arms at the expense of centromeric regions. Meiotic recombination rate in *MET1* × *met1* crosses vs wild type × wild type crosses was 75–100% higher in euchromatin than in heterochromatin, in former crosses as compared to latter crosses (Mirouze *et al.* 2012). In the wild type × *ddm1* cross as well the cross overs in the repeat rich centromeric regions of chromosomes were robustly suppressed (Colome-Tatche *et al.* 2012). Exposure to a variety of stress conditions that led to hypomethylation was observed to enhance the frequencies of mitotic and meiotic recombination in *A. thaliana* (table 8, rows 7 and 11). TMV infection on *Nicotiana tabacum* also increased the recombination rate (table 8, row 14). These observations have suggested that DNA methylation controls meiotic recombination. It appears that in the true breeding hypomethylated genetic backgrounds on one hand release of genetic variability via mutations is compromised but on the other hand it is stimulated via genetic recombination.

Negative regulation of transposition by DNA methylation

Transposable elements (TE) are DNA sequences that can replicate only when they are a part of their host genome. TE are abundantly present in plant genomes. They are the major form of repeat sequences that are preferentially located in the pericentromeric and centromeric regions of plant chromosomes. TE comprise 30%–90% of plant genomes, about 30% in the small genome of *A. thaliana* (140 Mbp), over 80% in the medium size genome of *Z. mays* (2700 Mbp) and about 90% in the large size genome of *Triticum aestivum* (17000 Mbp) (Vitte and Bennetzen 2006). Plant genomes increase in their size by TE expansion, polyploidization or both. *Oryza australiensis* genome has doubled its size by accumulating more than 90,000 copies of a *copia* type retrotransposon

Table 11. Activation/loss of repression in transposable elements (TE) and/or their transposability and amplification and well-known epimutations caused by their transpositions, resulting from the loss of genomewide DNA methylation in plants.

Transposable elements (TE)/TE family	Host	Condition(s) of TE activation	Reference(s)
Tto1 and Tar 17	*Arabidopsis thaliana*	*ddm1* mutational background	Hirochika *et al.* (2000)
CAC1	As above	As above	Miura *et al.* (2001)
Wis2-1A	*Aegilops sharonensis* × *Triticum monococcum* ssp *aegilopoides*	Nascent amphiploidy	Kashkush *et al.* (2003)
Tam3	*Arabidopsis thaliana*	Low temperature	Hashida *et al.* (2003)
Evade	As above	*ddm1* and *met1* mutational backgrounds	Mirouze *et al.* (2009)
Gypsy and Copia	As above	As above	Tsukahara *et al.* (2009)
ONSEN	As above	pol IV or *rdr2* mutational background or high temperature, associated with extensive genomic demethylation	Ito *et al.* (2011); Matsunaga *et al.* (2012)
Various transposons (TEs)	As above	The genes concerned with defence against pathogenic bacteria got primed by demethylation of TEs linked to the defence genes.	Yu *et al.* (2013)
RIKE, KONGOUROU, WALLAB1	*Oryza australiensis*	Exposure to radiation (?)	Piegu *et al.* (2006)
Ac/Ds	*Zea mays*	Wounding, pathogen attack or cold stress caused hypomethylation	Wessler (1996); Brutnell *et al.* (1997); Steward *et al.* (2000)
m Ping	*Oryza sativa*	Spontaneous	Naito *et al.* (2009)
Dasheng	As above	Spontaneous tissue specific expression led to expression of flanking genes via 3′ LTR promoter, such as *CRY1b* in roots, ISOPENTENYL-DIPHOSPHATE in leaves and rRNA METHYLASE in both leaves and roots.	Kashkush and Khasdan (2007)
LINE	*Arabidopsis thaliana*	Flanking of *BNS* gene by a long interspersed repeated element LINE leads to hypermethylation at BNS in *ddm1* mutant by a CMT3-KYP led methylation process leading to *bns* or dwarf phenotype	Sasaki *et al.* (2012a, b)
Gyno-hAT	*Cucumis melo*	Spread of methylation from a transposon of the *Gyno-hAT* family to the promoter of the *WIP1* transcription factor leads to degeneration of carpel primordia and a male flower develops from an original bisexual meristem	Martin *et al.* (2009)
MUTATOR	*Arabidopsis thaliana*	Insertion of MUTATOR into first intron of *FLC* gene in the *LER* strain delays flowering	Gazzani *et al.* (2003)

Table 11. (*contd.*)

Transposable elements (TE)/TE family	Host	Condition(s) of TE activation	Reference(s)
Tail-to-tail inverted repeat	As above	At the *PAI* locus (for a step in tryptophan biosynthesis) in the WS strain, of the four genes two (*PAI2* and *PAI3*) are unlinked and two (*PAI1* and *PAI4*) are arranged as tail-to-tail inverted repeats (IR). The siRNA synthesized from IR region hypermethylates promoters of all the four genes and thus PAI locus is silenced	Luff *et al.* (1999); Melquist *et al.* (1999)
Tandem repeats and a SINE	As above	DNA methylation of *FWA* gene promoter region, which contains two pairs of tandem repeats and a SINE (short interspersed repeat element), that occurs by the RdDM keeps *FWA* repressed. Its ectopic expression causes late flowering by inactivation of *FT* by FT × FWA interaction. Like the endogenous gene, *FWA* transgene is also kept inactivated by RdDM mediated promoter hypermethylation	Lippman *et al.* (2004); Chan *et al.* (2006a, b); Ikeda *et al.* (2007)
MuDR	*Zea mays*	MuDR transposon is silenced by siRNAs formed from inverted duplication of partially deleted MuDR by targeting of inverted terminal repeats of MuDR	Slotkin *et al.* (2005)

(Piegu *et al.* 2006). The integrity of plant genomes largely depend on the silencing of TE by DNA methylation (Lisch 2009).

Transposable elements can be retrotransposons or DNA elements (transposons). Whereas retrotransposons are infectious in that their progeny copies transpose to new locations without loss of the parental copy, the DNA elements excise from one location to integrate at another location, in host genome. Class I retrotransposons are characterized by their long terminal repeat (LTR) sequences. LTR at each end of retrotransposon has transcription termination and promoter regions, such that the 3′ promoter can read out the downstream host sequences. Class II retrotransposons have an internal promoter and occur in the form of long interspersed nuclear elements (*LINE*s) and short interspersed nuclear elements (*SINE*s). Retrotransposons can raise their number and inflate the host genome size over a very short evolutionary scale time (Lisch and Bennetzen 2011). Many TE present in plant genomes may have mutated such that they can no longer undergo transposition (Rangwala and Richards 2007). Defective TE may have come under the control of host genes (Jaenisch and Bird 2003; Rangwala and Richards 2007). Often LTR retrotransposon sequences occur in tandem. TE especially LTR TE serve as agents of genome restructuring. Illegitimate recombination events between homologous TE cause duplications, deletions, inversions and translocations (Vitte and Bennetzen 2006). All plant chromosomes have TE within them. Existence of TE in plant genomes may be symbiotically beneficial for both TE and plants.

TE are powerful mutagens. Plants protect their genomes against TE expansion and TE insertion caused mutations in host genome by methylating TE sequences. DNA methylation on TE on the one hand suppresses their transcription and thereby makes transposase (TPase) enzyme unavailable and on the other hand TPase access to TE is blocked. All of the cytosine methylation related pathways act on TE DNA such that cytosines throughout TE get methylated and methylation marks are maintained in CG, CHG and CHH contexts. TE occurring in natural plant populations have also been observed to be similarly heavily methylated (Vaughn *et al.* 2007). Generally, methylation intensity-wise, various types of plant genomic sequences can be arranged in the following order: protein coding genes < noncoding-repeat, pseudogene-repeat and protein-noncoding-repeat sequences < TE. Correct propagation of methylation marks through mitotic divisions allows normal plant development (Reinders *et al.* 2009).

In conditions that make the DNA methylation process defective, retrotransposons and DNA elements get transcriptionally activated. This happens in *met1*, *cmt3*, *pol IV*, *rdr2*, *ddm1* and other RdDM mutant backgrounds and on exposure to harsh environmental conditions such as high or low temperature, radiation, wounding, pathogen attack, etc. and polyploidization (Shaked *et al.* 2001; Tsukahara *et al.* 2009; Karan *et al.* 2012). Table 11 (rows 1–12) provides a list of several genetic backgrounds and environments that led to TE activation in different plant species.

Upon their activation, TE can cause transcriptional inactivation of host gene by inserting within it or alter expression of host gene by getting inserted adjacent to gene. Table 11 (rows 13–18) gives several examples of gene expression changes caused by TE insertions near to host genes. Figure 13 shows the mechanism whereby many host genes got inactivated by insertional mutagenesis of the TE *Wis2-1A* in a nascent amphiploid of wild-type wheat (Kashkush *et al.* 2003).

Some host genes are known to have acquired retrogenetic property from retrotransposons. This condition is exemplified by a cysteine-rich protein coding (*CRP*) gene. This *CRP* gene, much like retrotransposons, is very densely methylated

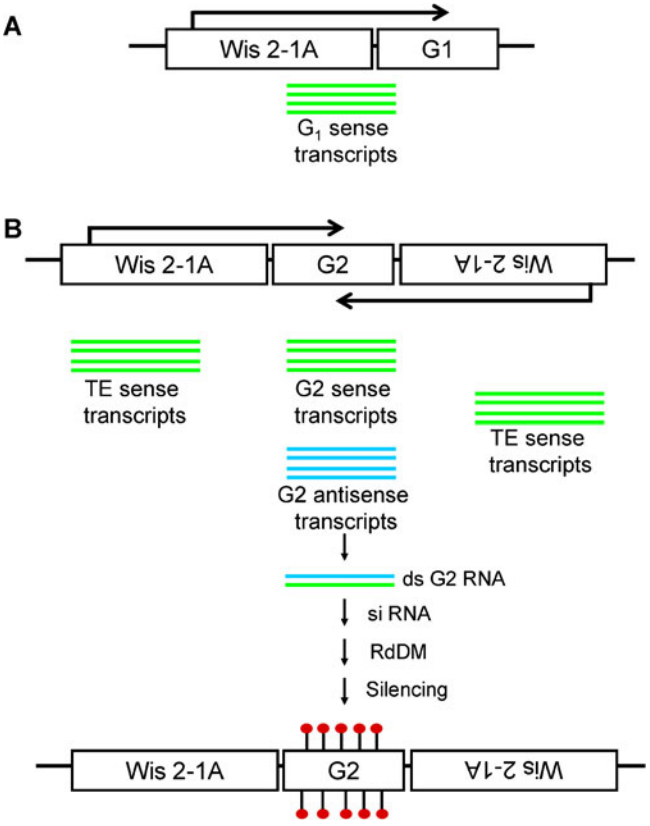

Figure 13. Transcriptional activation of long terminal repeat retrotransposons *Wis 2-1A* (TE) in synthetic amphiploid wheat leading to interference in the expression of coding genes. In the amphiploids of *Aegilops sharonensis* and *Triticum monococcum* ssp. *aegilopoides*, the TE *Wis 2-1A* gets activated due to pruning of their epigenetic marks. Their transcripts read out the neighbouring genes in the sense and antisense directions. Read outs result in activation of some coding genes and repression (silencing) of some other coding genes. (A), G1 gene is shown activated. (B), G2 gene is shown silenced via RNA-directed DNA methylation pathway, since G2 got expressed in both sense and antisense directions due to the activities of two *Wis 2-1A* TEs that were arranged in opposite directions on the two sides of G2.

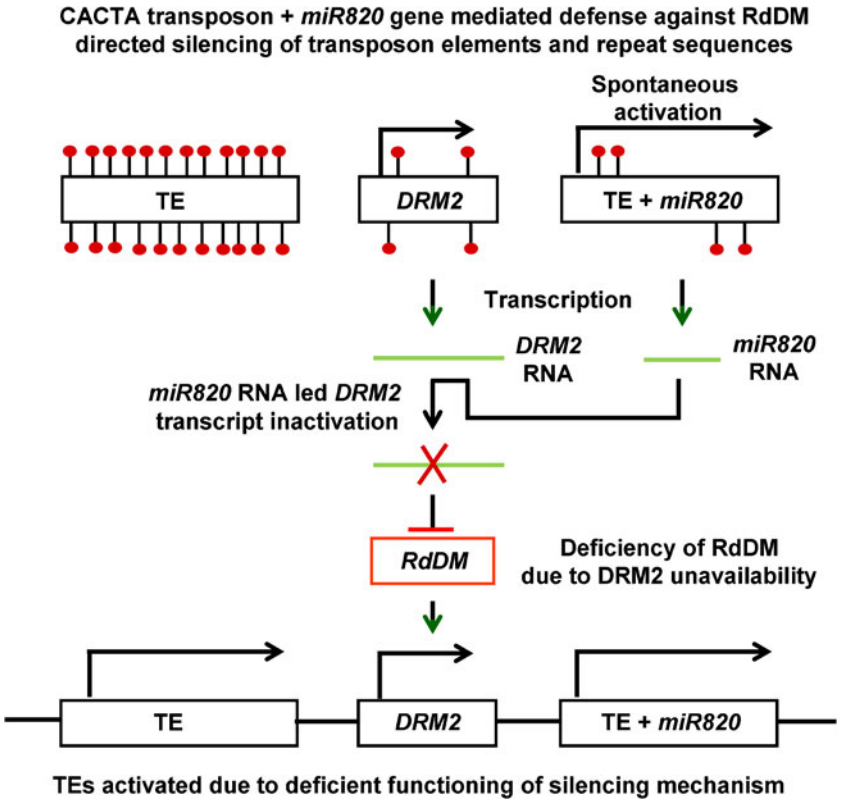

Figure 14. *Oryza sativa* genome has many copies of *CACTA* type transposon elements and five copies of one of them contain the gene for the miR820 precursor. The miR820 has homology for the *DRM2* gene whose product, the *de novo* DNA methyltransferase is an essential component of the RNA-directed DNA methylation (RdDM) pathway. The miR820 negatively regulates DRM2 expression via transcript degradation. Undersupply of DRM2 makes RdDM driven transcription silencing mechanism nonfunctional. As a result, transposons become active in expression and cause further changes in the gene network of the host.

in CG, CHG and CHH contexts. Based on DNA sequence in and around this gene, it is believed to have a retrogene origin that is via reverse transcription of mature mRNAs followed by insertion (You *et al.* 2012). This result suggests that some genes that occur in many copies in plant genomes may have similar origin. The observation suggests a mechanism for biotechnological gene dosage improvement of agriculturally important genes.

Several cultivated and wild rice species, including *O. sativa*, demonstrate extraordinary genome instability via TE expression. In certain *O. sativa* strains, it is possible to spontaneously obtain morphologically distinct TE tagged lines (Eun *et al.* 2012). In *O. sativa*, there has been disruption of the RdDM cytosine methylation pathway against the CACTA type retrotransposon TE. As shown in figure 14, several copies of *CACTA* TE have acquired within them a micro RNA gene *miR820* which has homology for the DRM2 cytosine methyltransferase gene of RdDM. Expression of miR820 provides defence to *CACTA* TEs against the host's TE silencing mechanism (Nosaka *et al.* 2012). Since the TE × *miR820* gene element got selected and propagated, and TE transpositions generate new gene regulatory network (Ito *et al.* 1999), it appears that the association between TE and host genome may indeed be symbiotic.

Inactive centromeres are hypomethylated

Each chromosome of the diploid ($2n$) genome of angiospermic plant species has one centromere. Generally this locus (centromere) is composed of a complex of tandem arrays of simple repeats and retrotransposons (Ma *et al.* 2007). Centromere serves as the kinetochore for the attachment of microtubules at mitosis and meiosis. It allows regular separation of chromatids at mitotic divisions and homologous chromosomes at the time of reduction division in meiosis. In some lines of *Z. mays* (maize), there is occurrence of chromosomes that possess two or more centromeres (Zhang *et al.* 2010; Koo *et al.* 2011; Fu *et al.* 2012). Molecular analysis of centromeres of the stable dicentric chromosomes revealed that one of the centromere on them was inactive. The inactivity was found related to hypomethylation of the dysfunctional centromere locus. Normal centromere was hypermethylated (Zhang *et al.* 2010; Fu *et al.* 2012).

How the methylation is differentially controlled at the two centromeres in stable dicentric chromosomes remains to be understood.

Cytosine methylation in DNA and three-dimensional arrangement of chromosomes in interphase nucleus

There is evidence that the arrangement of chromosomes with respect to nuclear polarity and each other is spatially fixed and a species characteristic in plants (Kumar and Natarajan 1966; Vogel and Schroeder 1974; Avivi and Feldman 1980; Coming 1980; Cremer *et al.* 1982). A fixed arrangement of chromosomes implies *cis* and *trans* interactions within and between chromosomes and that genes are nonrandomly positioned in the nucleus, such that some genes located on different chromosome may lie very near to each other in the nuclear space. The nonrandom three-dimensional arrangement of chromosomes may arise from the cytosine methylation patterns on noncoding repeat sequences, pseudogenes, rDNA repeats and TE. DNA methylation signal is read out by methyl-CpG-binding domain (MBD) proteins (Sasai and Defossez 2009). Several families of MBD proteins are known to recognize and maintain cytosine methylation marks by binding to them (Zemach and Grafi 2003). A class of MBD proteins have more than one MBD domain. Such MBDs may bind two different methylated CGs (Zemach and Grafi 2003, 2007). As a result of such interactions, genes located far apart will be brought together (figure 15).

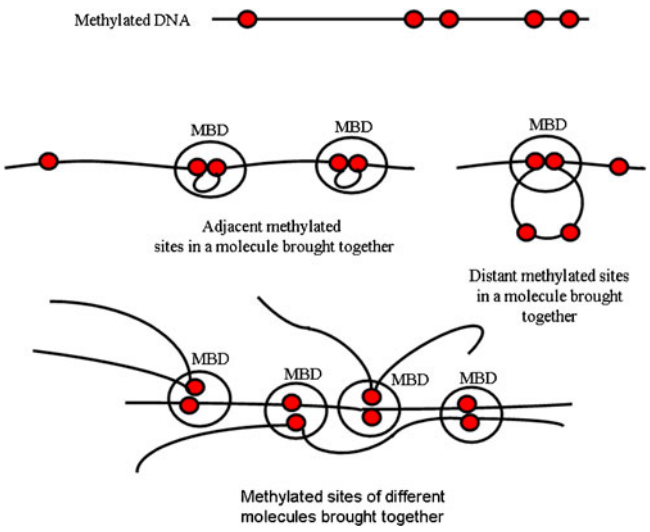

Figure 15. Spatial arrangement of chromatin modified by methyl-CpG-binding domain (MBD) protein (adapted after Kumar 2006). Since, several MBD domains may be present in them, certain MBD proteins can bring distant chromatin sites in proximity to each other. Such sites may be centres of coordinated repression or activation of transcription. MBD interactions with cytosines methylated sites are thought to be responsible for species specific three dimensional structure of chromosomes in nucleus.

These intrachromosomal and interchromosomal assemblies of genes may promote common controls; activation and repression of expression, binding of products for cofactorial action, coordination of DNA replication and promotion of illegitimate mitotic recombination.

Transgenerational inheritance of correlated changes in methylation marks and acquired traits supports Lamarckian concepts about evolution

Stress induced widespread DNA demethylation in genome and consequent acquirement of heritable defence against the stress has allowed combining of Darwinian and Lamarckian views about evolution (figure 16). The diverse views on evolution were synthesized in 1930s–1940s, as a result of the work of several biometrical-geneticists and systematist-naturalists, in the form of a theory which has come to be known as Neo-Darwinism (Mayr 1984). According to the Neo-Darwinism, the heritable resistance against a stress can arise by random mutation(s) in gene(s) that affect response to the stress followed by fixation of the favourable mutation(s) in population by selection. Contrastingly, Lamarck (Lamarck 1809) visualized that stress directly induced mutation(s) in the pertinent gene(s) such that they provided resistance/adaptation to stress in a heritable manner. These concepts are discussed below in the light of the current understanding of the dynamics of cytosine methylation in plants.

A common type of response of plants to certain stresses is widespread demethylation of genome. Thus, there is immediate adaptation due to changes in the cytosine methylation marks. These changes and associated stress adaptation will be heritable until the acquired epigenetic cytosine methylation marks are lost. Cytosine methylation marks are erased from coding genes as well as TE. Due to TE activation, transposon insertion mutations can occur, within genes and on the flanks of genes. Active demethylation can result in all kinds of point mutations and deletion and insertion mutations. Therefore, there will be chances of mutational evolvement of active alleles of gene(s) that impart adaptation stress via its/their involvement in the regulatory gene network. Occurrence of favourable genetic changes, upon selection in the stressed environment, will make the plant population adapted to stress for two reasons, epigenetic changes and genetic changes.

Epigenetical applications for improved agriculture

The present level of understanding of establishment, maintenance, loss, distribution, phenotypic effects and inheritance of cytosine methylation marks on DNA of plants is indicative of several kinds of applications for crop improvement. A list of possibilities that occurred to the authors is presented here. (i) Treatment of F_0 plants with specific chemicals for production of seeds to be used for cropping under abiotic

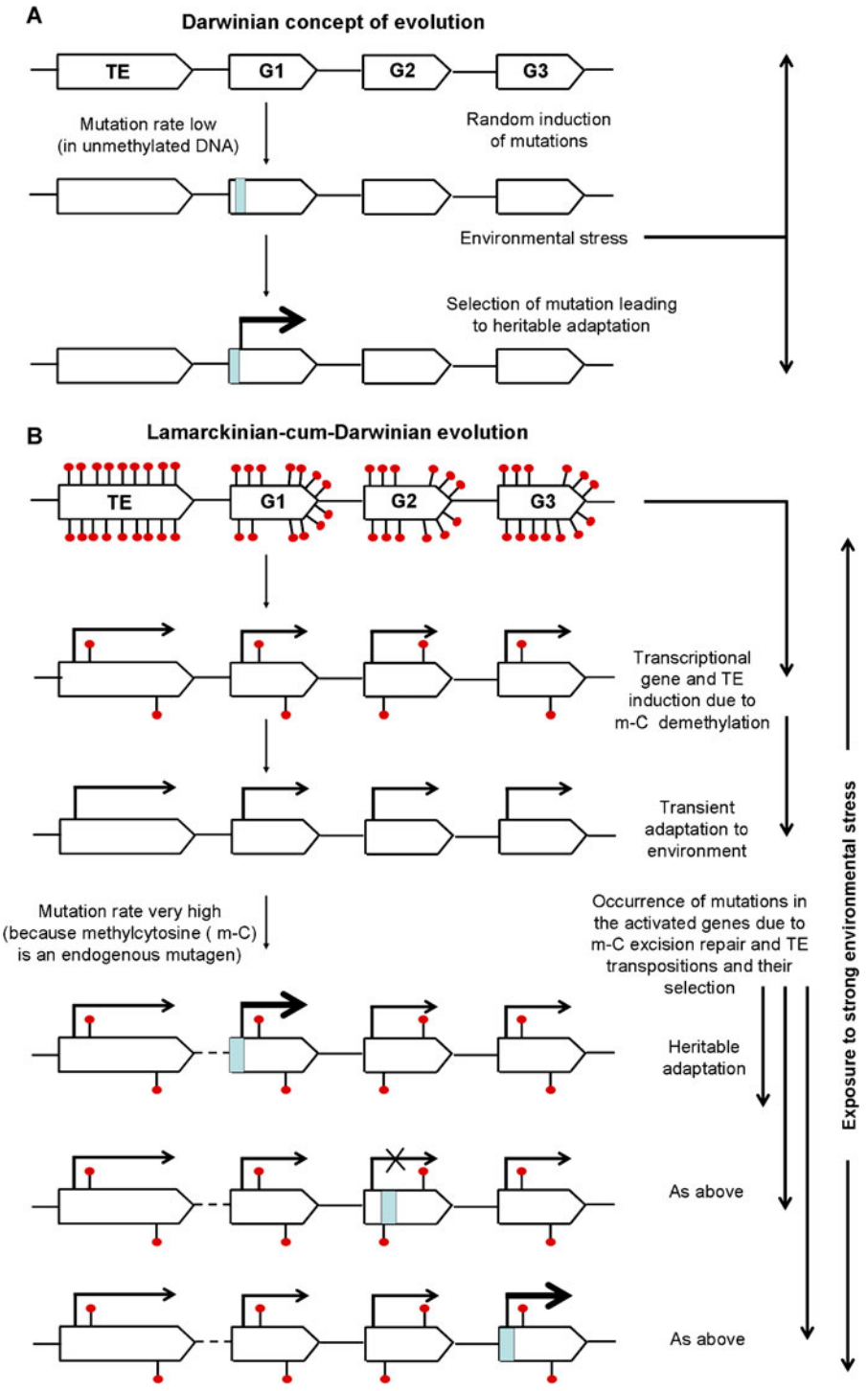

Figure 16. Diagramme depicting the Darwinian and Lamarck-cum-Darwinian modes of evolution for tolerance towards a stress in plants. (A), Darwin (1859) visualized that random undirected mutations enlarge the phenotypic variation in populations. Subsequently, selection of variants adapted to the prevailing environment fixes the favourable genetic changes in the population. (B), Lamarckian mode suggests that an environmental stress will become a cause for the induction of mutations in specific genes that will provide adaptation to the environmental stress. The available evidence on genetics of the epigenetic cytosine DNA methylation marks suggests applicability of Lamarckian-cum-Darwinian concept to evolution for adaptation to environment. Stress may lead to pruning of methylated cytosines from DNA, thus creating opportunities for induction of transitions, transversion, deletion and addition mutations in the demethylated genes, involved in providing protection against stress. Thus active alleles of genes that provide protection against stress may be created and subsequently selected. Mutations may also be caused in any of the genes involved in *de novo* or maintenance DNA methylation or active demethylation. These will be adaptive and subsequently the genome may select suppressive mutations balanced with creation of active allele in critical genes required for adaptation to stress. Evolution may be guided by the mechanisms visualized in Darwinian and Lamarckian concepts of generation of adaptive genetic changes.

and biotic stress conditions or for improved habit, branching and fruiting (chemical genetics). (ii) Construction of regular or conditional RNAi *MET1/DRM2/CMT3* transgenics or *met1/drm2/cmt3* mutants of corresponding orthologs in crop plants to: (a) improve recombination frequencies to break tight linkages and recover rare recombinants in small segregating populations; (b) produce apomictic seeds and triploid lines and (c) to recover viable rare fertile interspecific amphiploids, introgressed genotypes and transgenics and somatic hybrids. (iii) Combining of radiation or chemical mutagenic treatments with one or more stresses to obtain new alleles of already available gene mutants and for high efficiency mutagenesis. (iv) Increasing of copy number of specific genes such as for nutritional improvement of crop plants and productivity of industrial materials or to inactivate selected genes via construction of miRNAs. (v) Enhancement of somaclonal variation. (vi) Directed mutagenesis to generate active alleles of genes in pathways controlling flowering time, seed/fruit maturation time and other traits. (vii) To improve crop yield in F_0 generation.

Epigenetics is bound to promote biotechnological research in the budding area of chemical genetics. Evidence has been presented above (table 8) that treatment of plants with salicylic acid, jasmonic acid, 5-azacytidine or β-butyric acid leads to epigenetic alterations based systemic acquired resistance to biotic and/or abiotic stresses that is transgenerational. High throughput screening of chemical compound libraries has recently identified on the one hand compounds that lead to endogenous accumulation of salicylic acid (Noutoshi *et al.* 2012a, b and c) and on the other hand those that stimulated synthesis of nitric oxide (Floryszak-Wieczorek *et al.* 2012), both sets provided protection against herbivores, pathogens, salinity and/or drought. Further research should lead to identification of new chemicals and methods of their application such that they will provide protection to crops against a range of biotic and abiotic stresses. Since seeds produced on primed plants result in protected crops, the crop production will be economical and environmentally safe. It is thought that chemical genetics may also allow control of phenological features, ensuring productive crop rotations.

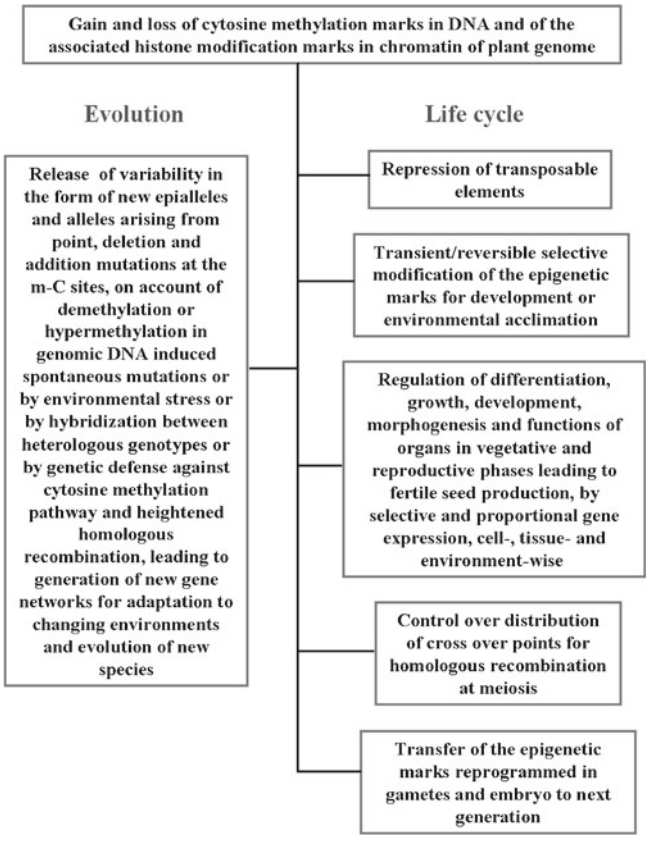

Figure 17. Inheritance and roles of epigenetic marks in plants.

Concluding remarks

The epigenetic cytosine methylation marks, formed *de novo* and maintained through mitotic and meiotic cell divisions, at CG, CHG and CHH elements in genome, comprise an important and conserved plant trait. Superimposition of methylome over genome leads to a multiplicative effect on phenotypic variability in plant populations, which face natural changes in temperature and daylength in seasons and occasional very harsh environmental conditions. Figure 17 broadly indicates the role of methylome in the life cycle and evolution in plants. The cytosine methylation caused epigenetic changes perhaps affect the plant life cycle omnifariously, by adapting cell division, tissue differentiation, organ development, flowering time, gametogenesis, embryogenesis, seed viability and dormancy and plant's response to seasons and other plant functions to their continuously changing environment. DNA methylation impacts plant functions via its regulatory effects on gene expression levels. Maintenance of genomic stability via repression of innate transposons and foreign plant/animal/pathogen DNA is another enormously important role of endowment of methylation mechanism in plants. DNA methylation dynamics control generation of genetic variability via determination of genetic recombination and mutation rates and thereby methylation mechanisms serve as an important driving force for evolution in angiosperms. Multiplicity in the pathways that establish or maintain one or the other kinds of methylation marks and linkages between methylation marking and chromatin marking genetic systems are suggestive of essentiality of DNA methylation for plant survival. Manipulation of plant methylome together with genetic variability offers vast, largely as yet unexplored, opportunities for the development of biotechnologies for better crop husbandry and for new kinds of varieties for yield and quality improvement of the produce. In view of a variety of unanswered questions and possible applications, methylomics will continue to be an important area of research in genetics.

Acknowledgments

Authors thank the Indian National Science Academy and the Council of Scientific and Industrial Research, Government of India, for the scientistship schemes to SK, and Department of Biotechnology and SKA Institution for Research, Education and Development for Postgraduate fellowships respectively to RK and VS.

References

Agboola R. S., Kimatu J. N., Liao Y. C. and Liu B. 2012 Morphological and cytosine DNA methylation changes induced by combined effect of boron (B) and salt toxicity in *Sorghum bicolor* inbred line. *Afr. J. Biotech.* **11**, 10874–10881.

Agius F., Kapoor A. and Zhu J. K. 2006 Role of the *Arabidopsis* DNA glycosylases/lyase ROS1 in active DNA demethylation. *Proc. Natl. Acad. Sci. USA* **103**, 11796–11801.

Akimoto K., Katakami H., Kim H. J., Ogawa E., Sano C. M., Wada Y. and Sano H. 2007 Epigenetic inheritance in rice plants. *Ann. Bot.* (Lond.) **100**, 205–217.

Alina S., Sgorbati S., Santagostino A., Labra M., Ghiani A. and Citterio S. 2004 Specific hypomethylation of DNA is induced by heavy metals in white clover and industrial hemp. *Physiol. Plant* **121**, 472–480.

Alleman M., Sidorenko L., McGinnis K., Seshadri V., Dorweiler J. E., White J. *et al.* 2006 An RNA-dependent RNA polymerase is required for paramutation in maize. *Nature* **442**, 295–298.

Arber W. and Linn S. 1969 DNA modification and restriction. *Annu. Rev. Biochem.* **38**, 467–500.

Aufsatz W., Mette M. F., van der Winden J., Matzke M. and Matzke A. J. 2002 HDA6, a putative histone deacetylase needed to enhance DNA methylation induced by double-stranded RNA. *EMBO J.* **21**, 6832–6841.

Aufsatz W., Mette M. F., Matzke A. J. and Matzke M. 2004 The role of *MET1* in RNA-directed *de novo* and maintenance methylation of CG dinucleotides. *Plant Mol. Biol.* **54**, 793–804.

Ausin I., Mockler T. C., Chory J. and Jacobsen S. E. 2009 *IDN1* and *IDN2* are required for *de novo* DNA methylation in *Arabidopsis thaliana*. *Nat. Struct. Mol. Biol.* **16**, 1325–1327.

Avivi L. and Feldman M. 1980 Arrangement of chromosomes in the interphase nucleus of plants. *Hum. Genet.* **55**, 281–295.

Bairu M. W., Aremu A. O. and van Staden J. 2011 Somaclonal variation in plants: causes and detection methods. *Plant Growth Regul.* **63**, 147–173.

Bauer M. J. and Fischer R. L. 2011 Genome demethylation and imprinting in the endosperm. *Curr. Opin. Plant Biol.* **14**, 162–167.

Becker C., Hagmann J., Muller J., Koenig D., Stegle O., Borgwardt K. and Weigel D. 2011 Spontaneous epigenetic variation in the *Arabidopsis thaliana* methylome. *Nature* **480**, 245–249.

Berger S. L. 2007 The complex language of chromatin regulation during transcription. *Nature* **447**, 407–412.

Berger F. and Twell D. 2011 Germline specification and function in plants. *Ann. Rev. Plant Biol.* **62**, 461–484.

Bernstein B. E., Meissner A. and Lander E. S. 2006 The mammalian epigenome. *Cell* **128**, 669–681.

Bhutani N., Burns D. M. and Blau H. M. 2011 DNA demethylation dynamics. *Cell* **146**, 866–872.

Bies-Etheve N., Pontier D., Lahmy S., Picart C., Vega D., Cooke R. and Lagrange T. 2009 RNA-directed DNA methylation requires an AGO4-interacting member of SPT5 elongation factor. *EMBO Rep.* **10**, 649–654.

Billichak A., Ilnystkyy Y., Hollunder J. and Kovalchuk I. 2012 The progeny of *Arabidopsis thaliana* plants exposed to salt exhibit changes in DNA methylation, histone modifications and gene expression. *PLoS ONE* **7**, e30515.

Boyko A., Kathiria P., Zemp F. J., Yao Y., Pogribny I. and Kovalchuk I. 2007 Transgenerational changes in the genome stability and methylation in pathogen-infected plants. *Nucleic Acids Res.* **35**, 1714–1725.

Boyko A., Blevins T., Yao Y., Golubov A., Bilichak A., Ilnystkyy Y. *et al.* 2010 Transgenerational adaptation of *Arabidopsis* to stress requires DNA methylation and the functions of dicer-like proteins. *PLoS ONE* **5**, e9514.

Brenet F., Moh M., Funk P., Feierstein F., Viale A. J., Socci N. D. and Scandura J. M. 2011 DNA methylation of the first exon is tightly linked to transcriptional silencing. *PLoS ONE* **6**, e14524.

Brown J. C. L., De Decker M. M. and Fieldes M. A. 2008 A comparative analysis of developmental profiles for DNA methylation in 5-azacytidine-induced early flowering flax lines and their control. *Plant Sci.* **175**, 217–225.

Brutnell T. P., May B. P. and Dellaporta S. L. 1997 The *Ac-st2* element of maize exhibits a positive dosage effect and epigenetic regulation. *Genetics* **147**, 823–834.

Brzeski J. and Jerzmanowski A. 2003 Deficient in DNA methylation 1 (DDM1) defines a noval family of chromatin remodelling factors. *J. Biol. Chem.* **278**, 823–828.

Calarco J. P., Borges F., Donoghue M. T., Van Ex F., Jullien P. E., Lopes T. *et al.* 2012 Reprogramming of DNA methylation in pollen guides epigenetic inheritance via small RNA. *Cell* **151**, 194–205.

Calonji M. and Sung Z. R. 2006 Complexity beneath the silence. *Curr. Opin. Plant Biol.* **9**, 530–537.

Cao X. and Jacobsen S. E. 2002a Locus specific control of asymmetric and CpNpG methylation by *DRM* and *CMT3* methyltransferase genes. *Proc. Natl. Acad. Sci. USA* **99**, 16491–16498.

Cao X. and Jacobsen S. E. 2002b Role of the *Arabidopsis DRM* methyltransferase in *de novo* DNA methylation and gene silencing. *Curr. Biol.* **12**, 1138–1144.

Cao X., Aufsatz W., Zilberman D., Mette M. F., Huang M. S., Matzke M. and Jacobsen S. E. 2003 Role of *DRM* and *CMT3* methyltransferases in RNA-directed DNA methylation. *Curr. Biol.* **13**, 2212–2217.

Cao D., Gao X., Liu J., Kimatu J. N., Geng S., Wang X. *et al.* 2011 Methylation sensitive amplified polymorphism (MSAP) reveals that alkali stress triggers more DNA hypomethylation levels in cotton (*Gossypium hirsutum* L.) roots than salt stress. *Afr. J. Biotech.* **10**, 18971–18980.

Chan S. W., Henderson I. R. and Jacobsen S. E. 2005 Gardening the genome DNA methylation in *Arabidopsis thaliana*. *Nat. Rev. Genet.* **6**, 351–360.

Chan S. W., Henderson I. R., Zhang X., Shah G., Chien J. S. and Jacobsen S. E. 2006a RNAi, DRD1 and histone methylation actively target developmentally important non-CG DNA methylation in *Arabidopsis*. *PLoS Genet.* **2**, e83.

Chan S. W., Zhang X., Bernatavichute Y. V. and Jacobsen S. E. 2006b Two-step recruitment of RNA-directed DNA methylation to tandem repeats. *PLoS Biol.* **4**, e363.

Chandler V. L. 2007 Paramutation: from maize to mice. *Cell* **128**, 641–645.

Choi C. S. and Sano H. 2007 Abiotic-stress induces demethylation and transcriptional activation of a gene encoding a glycerophosphodiesterase-like protein in tobacco plants. *Mol. Genet. Genomics* **277**, 589–600.

Choi Y., Gehring M., Johnson L., Hannon M., Harada J. J., Goldberg R. B. *et al.* 2002 DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in *Arabidopsis*. *Cell* **110**, 33–42.

Chuck G. and O'Connor D. 2010 Small RNAs going the distance during plant development. *Curr. Opin. Plant Biol.* **13**, 40–45.

Colome-Tatche M., Cortijo S., Wardenaar R., Morgado L., Lahouze B., Sarazin A. *et al.* 2012 Features of the *Arabidopsis* recombination landscape resulting from the combined loss of sequence variation and DNA methylation. *Proc. Natl. Acad. Sci. USA* **109**, 16240–16245.

Coming D. E. 1980 Arrangement of chromatin in the nucleus. *Hum. Genet.* **53**, 131–143.

Conde D., Gonzalez-Melendi P. and Allona I. 2013 Poplar stems show opposite epigenetic patterns during winter dormancy and vegetative growth. *Trees* **27**, 311–320.

Cremer T., Cremer C., Schneider T., Baumann H., Hens L. and Kirsch-Volders M. 1982 Analysis of chromosome positions in the interphase nucleus of Chinese hamster cells by laser UV-microirradiation experiments. *Hum. Genet.* **62**, 201–209.

Cubas P., Vincent C. and Coen E. 1999 An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* **401**, 157–161.

Dalakouras A., Dadami E., Zwiebel M., Krczal G. and Wassenegger M. 2012 Transgenerational maintenance of transgene body CG but not CHG and CHH methylation. *Epigenetics* **7**, 1071–1078.

Darwin C. 1859 *On the origin of species*, 1st edition. Murray, London, UK.

Deal R. B. and Henikoff S. 2011 Histone variants and modifications in plant gene regulation. *Curr. Opin. Plant Biol.* **14**, 116–122.

Dowen R. H., Pelizzola M., Schmitz R. J., Lister R., Dowen J. M., Nery J. R. *et al.* 2012 Widespread dynamic DNA methylation in response to biotic stress. *Proc. Natl. Acad. Sci. USA* **109**, E2183–E2191.

Duan C. G., Fang Y. Y., Zhou B. J., Zhao J. H., Hou W. N., Zhu H. *et al.* 2012 Suppression of *Arabidopsis* ARGONAUTE 1-mediated slicing, transgene-induced RNA silencing, and DNA methylation by distinct domains of the Cucumber mosaic virus 2b proteins. *Plant Cell* **24**, 259–274.

Dyachenko O. V., Zakharchenko N. S., Shevchuk T. V., Bohnert H. J., Cushman J. C. and Buryanov Y. I. 2006 Effect of hypermethylation of CCWGG sequences in DNA of *Mesembryanthemum crystallinum* plants on their adaptation to salt stress. *Biochemistry* (Moscow) **71**, 461–465.

El-Shami M., Pontier D., Lahmy S., Braun L., Picart C., Vega D. *et al.* 2007 Reiterated WG/GW motifs from functionally and evolutionarily conserved ARGONAUTE-binding platforms in RNAi-related components. *Genes Dev.* **21**, 2539–2544.

Erhard Jr K. F., Stonaker J. L., Parkinson S. E., Lim J. P., Hale C. J. and Hollick J. B. 2009 RNA polymerase IV functions in paramutation in *Zea mays*. *Science* **323**, 1201–1205.

Eun C. H., Takagi K., Park K. I., Maekawa M., Iida S. and Tsugano K. 2012 Activation and epigenetic regulation of DNA transposon *nDart1* in rice. *Plant Cell Physiol.* **53**, 857–868.

Fang J. G. and Chao C. T. 2007 Methylation-sensitive amplification polymorphism in date palms (*Phoenix dactylifera* L.) and their off-shoots. *Plant Biol.* (Stuttg) **9**, 526–533.

Feng S., Jacobsen S. E. and Reik W. 2010 Epigenetic reprogramming in plant and animal development. *Science* **330**, 622–627.

Finnegan E. J., Peacock W. J. and Dennis E. S. 1996 Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. *Proc. Natl. Acad. Sci. USA* **93**, 8449–8454.

Fitz Gerald J. N., Hui P. S. and Berger F. 2009 Polycomb group-dependent imprinting of the actin regulator AtFH5 regulates morphogenesis in *Arabidopsis thaliana*. *Development* **136**, 3399–3404.

Floryszak-Wieczorek J., Arasimowicz-Jelonek M., Milczarek G., Janus L., Pawlak-Sprada S., Abramowskai D. *et al.* 2012 Nitric oxide-mediated stress imprints in potato as an effect of exposure to a priming agent. *Mol. Plant Microbe Interact.* **25**, 1469–1477.

Fu S., Gao Z., Birchler J. and Han F. 2012 Dicentric chromosome formation and epigenetics of centromere formation in plants. *J. Genet. Genomics* **39**, 125–130.

Fuchs J., Demidov D., Houben A. and Schubert I. 2006 Chromosomal histone modification patterns—from conservation to diversity. *Trends Plant Sci.* **11**, 199–208.

Gao Z., Liu H. L., Daxinger L., Pontes O., He X., Qian W. *et al.* 2010 An RNA polymerase II- and AGO4-associated protein acts in RNA-directed DNA methylation. *Nature* **465**, 106–109.

Garcia-Aguilar M., Michand C., Leblanc O. and Grimanelli D. 2010 Inactivation of a DNA methylation pathway in maize reproductive organs results in apomixis-like phenotypes. *Plant Cell* **22**, 3249–3667.

Gazzani S., Gendall A. R., Lister C. and Dean C. 2003 Analysis of the molecular basis of flowering time variation in *Arabidopsis* accessions. *Plant Physiol.* **132**, 1107–1114.

Gehring M., Bubb K. L. and Henikoff S. 2009 Extensive demethylation of repetitive elements during seed development underlies gene imprinting. *Science* **324**, 1447–1451.

Gehring M., Huh J. H., Hsieh T. F., Penterman J., Choi Y., Harada J. J. *et al.* 2006 DEMETER DNA glycosylase establishes MEDEA polycomb gene self-imprinting by allele-specific demethylation. *Cell* **124**, 495–506.

Gehring M., Missirian V. and Henikoff S. 2011 Genomic analysis of parent-of-origin allelic expression in *Arabidopsis thaliana* seeds. *PLoS ONE* **6**, e23687.

Gong Z., Morales-Ruiz T., Ariza R. R., Roldan-Arjona T., David L. and Zhu J. K. 2002 ROS1, a repressor of transcriptional gene silencing in *Arabidopsis*, encodes a DNA glycosylase/lyase. *Cell* **111**, 803–814.

Greenberg M. V., Ausin I., Chan S. W., Cokus S. J., Cuperus J. T., Feng S. *et al.* 2011 Identification of genes required for *de novo* DNA methylation in *Arabidopsis*. *Epigenetics* **6**, 344–354.

Grewal S. I. and Elgin S. C. 2007 Transcription and RNA interference in the formation of heterochromatin. *Nature* **447**, 399–406.

Gutierrez-Marcos J. F. and Dickinson H. G. 2012 Epigenetic reprogramming in plant reproductive lineages. *Plant Cell Physiol.* **53**, 817–823.

Haag J. R. and Pikaard C. S. 2011 Multisubunit RNA polymerases IV and V: purveyors of non-coding RNA for plant gene silencing. *Nat. Rev. Mol. Cell Biol.* **12**, 483–492.

Hashida S. N., Kitamura K., Mikami T. and Kishima Y. 2003 Temperature shift coordinately changes the activity and the methylation state of transposon Tam3 in *Antirrhinum majus*. *Plant Physiol.* **132**, 1207–1216.

Hashida S. N., Uchiyama T., Martin C., Kishima Y., Sano Y. and Mikami T. 2006 The temperature-dependent change in methylation of the *Antirrhinum* transposon Tam3 is controlled, by the activity of its transposase. *Plant Cell* **18**, 104–118.

He X. J., Hsu Y. F., Pontes O., Zhu J., Lu J., Bressan R. A. *et al.* 2009 NRPD4, a protein related to the RPB4 subunit of RNA polymerase II, is a component of RNA polymerase IV and V and is required for RNA-directed DNA methylation. *Genes Dev.* **23**, 318–330.

He G., Elling A. A. and Deng X. W. 2011 The epigenome and plant development. u*Annu. Rev. Plant Biol.* **62**, 411–435.

Henderson I. R., Zhang X., Lu C., Johnson L., Meyers B. C., Green P. J. and Jacobsen S. E. 2006 Dissecting *Arabidopsis thaliana* DICER function in small RNA processing, gene silencing and DNA methylation patterning. *Nature Gen.* **38**, 721–725.

Henderson I. R. and Jacobsen S. E. 2007 Epigenetic inheritance in plants. *Nature* **447**, 418–424.

Henderson I. R. and Jacobsen S. E. 2008 Tandem repeats upstream of the *Arabidopsis* SDC recruit non-CG DNA methylation and initiate siRNA spreading. *Genes Dev.* **22**, 1597–1606.

Henderson I. R., Deleris A., Wong W., Zhong X., Chin H. G., Horwitz G. A. *et al.* 2010 The *de novo* cytosine methyltransferase DRM2 requires intact UBA domains and a catalytically

mutated paralog DRM3 during RNA-directed DNA methylation in *Arabidopsis thaliana*. *PLoS Genet.* **6**, e1001182.

Henikoff S., Ahmad K. and Malik H. S. 2001 The centromere paradox: stable inheritance with rapidly evolving DNA. *Science* **293**, 1098–1102.

Herr A. J., Jensen M. B., Dalmay T. and Baulcombe D. C. 2005 RNA polymerase IV directs silencing of endogenous DNA. *Science* **308**, 118–120.

Hirochika H., Okamoto H. and Kakutani T. 2000 Silencing of retrotransposons in *Arabidopsis* and reactivation by the ddm1 mutation. *Plant Cell* **12**, 357–369.

Holliday R. and Pugh J. E. 1975 DNA modification mechanism and gene activity during development. *Science* **187**, 226–232.

Hsieh T. F., Ibarra C. A., Silva P., Zemach A., Eshed-Williams L., Fischer R. L. and Zilberman D. 2009 Genome-wide demethylation of *Arabidopsis* endosperm. *Science* **324**, 1451–1454.

Hsieh T. F., Shin J., Uzawa R., Silva P., Cohen S., Bauer M. J. *et al.* 2011 Regulation of imprinted gene expression in *Arabidopsis* endosperm. *Proc. Natl. Acad. Sci. USA* **108**, 1755–1756.

Huang L., Jones A. M., Searle I., Patel K., Vogler H., Hubner N. C. and Baulcombe D. C. 2009 An atypical RNA polymerase involved in RNA silencing shares small subunits with RNA polymerase II. *Natl. Struct. Mol. Biol.* **16**, 91–93.

Huff J. T. and Zilberman D. 2012 Regulation of biological accuracy, precision, and memory by plant chromatin organization. *Curr. Opin. Genet. Dev.* **22**, 132–138.

Ibarra C. A., Feng X., Schoft V. K., Hsieh T. F., Uzawa R., Rodrigues J. A. *et al.* 2012 Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. *Science* **337**, 1360–1364.

Ikeda Y. 2012 Plant imprinted genes identified by genome-wide approaches and their regulatory mechanisms. *Plant Cell Physiol.* **53**, 809–816.

Ikeda Y., Kobayashi Y., Yamaguchi A., Abe M. and Araki T. 2007 Molecular basis of late-flowering phenotype caused by dominant epi-alleles of the FWA locus in *Arabidopsis*. *Plant Cell Physiol.* **48**, 205–220.

Inagaki S., Miura-Kamio A., Nakamura Y., Lu F., Cui X., Cao X. *et al.* 2010 Autocatalytic differentiation of epigenetic modifications within the *Arabidopsis* genome. *EMBO J.* **29**, 3496–3506.

Ito T., Seki M., Hayashida N., Shibata D. and Shinozaki K. 1999 Regional insertional mutagenesis of genes on *Arabidopsis thaliana* chromosome using the *Ac/Ds* transposition in combination with cDNA scanning method. *Plant J.* **17**, 433–444.

Ito H., Gaubert H., Bucher E., Mirouze M., Vaillant I. and Paszkowski J. 2011 An siRNA pathway prevents transgenerational retrotransposition in plants subjected to stress. *Nature* **472**, 115–119.

Jackson J. P., Lindroth A. M., Cao X. and Jacobsen S. E. 2002 Control of CpNpG methylation by the KRYPTONYTE histone H3 methyltransferase. *Nature* **416**, 556–560.

Jaenisch R. and Bird A. 2003 Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet.* **33**, 245–254.

Jahnke S. and Scholten S. 2009 Epigenetic resetting of a gene imprinted in plant embryos. *Curr. Biol.* **19**, 1677–1681.

Jaligot E., Beule T., Baurens F. C., Billotte N. and Rival A. 2004 Search for methylation-sensitive amplification polymorphisms associated with the "mantled" variant phenotype in oil palm (*Elaeis guineensis* Jacq.) *Genome* **47**, 224–228.

Jiang H. and Kohler C. 2012 Evolution, function and regulation of genomic imprinting in plant seed development. *J. Exp. Bot.* **63**, 4713–4722.

Johannes F., Porcher E., Teixeira F. K., Saliba-Colombani V., Simon M., Agier N. *et al.* 2009 Assessing the impact of transgenerational epigenetic variation on complex traits. *PLoS Genet.* **5**, e1000530.

Johnson T. B. and Coghill R. D. 1925 Researches on pyrimidines CIII. The discovery of 5-methylcytosine in tuberculinic acid, the nucleic acid of the tubercle bacillus. *J. Am. Chem. Soc.* **47**, 2838–2844.

Johnson L. M., Bostick M., Zhang X., Kraft E., Henderson I., Callis J. and Jacobsen S. E. 2007 The SRA-methyl-cytosine-binding domain links DNA and histone methylation. *Curr. Biol.* **17**, 379–384.

Johnson L. M., Law J. A., Khattar A., Henderson I. R. and Jacobsen S. E. 2008 SRA-domain proteins required for DRM2-mediated *de novo* DNA methylation. *PLoS Genet.* **4**, e1000280.

Jullien P. E. and Berger F. 2008 Parental genomic imprinting in plants: significance for reproduction. *Med. Sci.* **24**, 753–757.

Jullien P. E. and Berger F. 2010 Parental genome dosage imbalance deregulates imprinting in *Arabidopsis*. *PLoS Genet.* **6**, e1000885.

Jullien P. E., Kinoshita T., Ohad N. and Berger F. 2006 Maintenance of DNA methylation during the *Arabidopsis* life cycle is essential for parental imprinting. *Plant Cell* **18**, 1360–1372.

Jullien P. E., Susaki D., Yelagandula R., Higashiyama T. and Berger F. 2012 DNA methylation dynamics during sexual reproduction in *Arabidopsis thaliana*. *Curr. Biol.* **22**, 1825–1830.

Kakutani T., Jeddeloh J. A., Flowers S. K., Munakata K. and Richards E. J. 1996 Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. *Proc. Natl. Acad. Sci. USA* **29**, 12406–12411.

Kakutani T., Kato M., Kinoshita T. and Miura A. 2004 Control of development and transposon movement by DNA methylation in *Arabidopsis thaliana*. *Cold Spring Harb. Symp. Quant. Biol.* **69**, 139–143.

Kankel M. W., Rasey D. E., Stoke T. L., Flowers S. K., Haag J. R., Jeddeloh J. A. *et al.* 2003 *MET1* cytosine methyltransferase mutants. *Genetics* **163**, 1109–1122.

Kanno T. and Habu Y. 2011 siRNA-mediated chromatin maintenance and its function in *Arabidopsis thaliana*. *Biochim. Biophys. Acta* **1809**, 444–451.

Kanno T., Mette M. F., Kreil D. P., Aufsatz W., Matzke M. and Matzke A. J. 2004 Involvement of putative SNF2 chromatin modelling protein DRD1 in RNA-directed DNA methylation. *Curr. Biol.* **14**, 801–805.

Kanno T., Bucher E., Daxinger L., Huettel B., Bohmdorfer G., Gregor W. *et al.* 2008 A structural-maintenance-of-chromosomes hinge domain-containing protein is required for RNA-directed DNA methylation. *Nat. Genet.* **40**, 670–675.

Kanno T., Bucher E., Daxinger L., Huettel B., Kreil D. P., Breinig F. *et al.* 2010 RNA-directed DNA methylation and plant development require an IWR1-type transcription factor. *EMBO Rep.* **11**, 65–71.

Karan R., DeLeon T., Biradar H. and Subudhi P. K. 2012 Salt stress induced variation in DNA methylation pattern and its influence on gene expression in contrasting rice genotypes. *PLoS ONE* **7**, e40203.

Kashkush K. and Khasdan V. 2007 Large-scale survey of cytosine methylation of retrotransposons and the impact of readout transcription from long terminal repeats on expression of adjacent rice genes. *Genetics* **177**, 1975–1985.

Kashkush K., Feldman M. and Levy A. A. 2003 Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. *Nat. Genet.* **33**, 102–106.

Kathiria P., Sidler C., Golubov A., Kalischuk M., Kowchuk L. M. and Kovalchuk I. 2010 Tobacco mosaic virus infection results in an increase in recombination frequency and resistance to viral, bacterial and fungal pathogen in the progeny of infected plants. *Plant Physiol.* **153**, 1859–1870.

Katiyar-Agarwal S., Morgan R., Dahlbeck D., Borsani O., Villegas Jr A., Zhu J. K. *et al.* 2006 A pathogen-inducible

endogenous siRNA in plant immunity. *Proc. Natl. Acad. Sci. USA* **103**, 18002–18007.

Kato M., Miura A., Bender J., Jacobsen S. E. and Kakutani T. 2003 Role of CG and non-CG methylation in immobilization of transposons in *Arabidopsis*. *Curr. Biol.* **13**, 421–426.

Kim J. M., To T. K., Ishida J., Morosawa T., Kawashima M., Matsui A. *et al.* 2008 Alterations of lysine modifications on histone H3 N-tail under drought stress conditions in *Arabidopsis thaliana*. *Plant Cell Physiol.* **49**, 1580–1588.

Kim J. M., To T. K., Ishida J., Matsui A., Kimura H. and Seki M. 2012 Transition of chromatin status during the process of recovery from drought stress in *Arabidopsis thaliana*. *Plant Cell Physiol.* **53**, 847–856.

Kinoshita T., Yadagiri R., Harada J. J., Goldberg R. B. and Fischer R. L. 1999 Imprinting of the MEDEA polycomb gene in the *Arabidopsis* endosperm. *Plant Cell* **11**, 1945–1952.

Kinoshita T., Miura A., Kinoshita Y., Cao X., Jacobsen S. E., Fischer R. L. and Kakutani T. 2004 One-way control of FWA imprinting in *Arabidopsis* endosperm by DNA methylation. *Science* **303**, 521–523.

Kinoshita Y., Saze H., Kinoshita T., Miura A., Soppe W. J., Koornneef M. and Kakutani T. 2007 Control of *FWA* gene silencing in *Arabidopsis thaliana* by SINE-related direct repeats. *Plant J.* **49**, 38–45.

Kohler C., Page D. R., Gagliardini V. and Grossniklaus U. 2005 The *Arabidopsis thaliana* MEDEA polycomb-group protein controls expression of *PHERES 1* by parental imprinting. *Nat. Genet.* **37**, 28–30.

Koltunow A. M. and Grossniklaus V. 2003 Apomixis: a developmental perspective. *Ann. Rev. Plant Biol.* **54**, 547–574.

Koo D. H., Han F., Birchler J. A. and Jiang J. 2011 Distinct DNA methylation patterns associated with active and inactive centromeres of the maize B chromosome. *Genome Res.* **21**, 908–914.

Kou H. P., Li Y., Song X. X., Ou X. F., Xing S. C., Ma J. *et al.* 2011 Heritable alteration in DNA methylation induced by nitrogen-deficiency stress accompanies enhanced tolerance by progenies to the stress in rice (*Oryza sativa* L.). *J. Plant Physiol.* **168**, 1685–1693.

Kouzarides T. 2007 Chromatin modifications and their function. *Cell* **128**, 693–705.

Kovalchuk O., Burke P., Arkhipov A., Kuchma N., James S. J., Kovalchuk I. and Pogribny I. 2003 Genome hypermethylation in *Pinus silvestris* of Chernobyl–a mechanism for radiation adaptation. *Mut. Res.* **529**, 13–20.

Kumar S. 2006 Progress in understanding of the genetic phenomenon paramutation. *Curr. Sci.* **91**, 1002–1003.

Kumar S. 2007 Mechanisms of heritable epigenetic variation mediated by cytosine methylation of nuclear genome in plants. *Proc. Natl. Acad. Sci. India* **77**, 19–30.

Kumar S. and Natarajan A. T. 1966 Kinetics of two-break chromosome exchanges and the spatial arrangement of chromosome strands in interphase nucleus. *Nature* **209**, 796–797.

Kumar S. V. and Wigge P. A. 2010 H2A.Z-containing nucleosomes mediate the thermosensory response in *Arabidopsis*. *Cell* **140**, 136–147.

Labra M., Ghiani A., Citterio S., Sgorbati S., Sala F., Vannini C. *et al.* 2002 Analysis of cytosine methylation pattern in response to water deficit in pea root tips. *Plant Biol.* **4**, 694–699.

Lamarck J. B. 1809 Philosophie zoologique., ou exposition des considerations relatives a l'histoire naturelle des animaux. Paris: Dentu.

Latzel V., Zhang Y. Y., Moritz K. K., Fischer M. and Bossdorf O. 2012 Epigenetic variation in plant responses to defence hormones. *Ann. Bot.* **110**, 1423–1428.

Lauria M. and Rossi V. 2011 Epigenetic control of gene regulation in plants. *Biochim. Biophys. Acta* **1809**, 369–378.

Law J. A. and Jacobsen S. E. 2010 Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat. Rev. Genet.* **11**, 204–220.

Law J. A., Ausin I., Johnson L. M., Vashisht A. A., Zhu J. K., Wohlschlegel J. A. and Jacobsen S. E. 2010 A protein complex required for polymerase V transcripts and RNA-directed DNA methylation in *Arabidopsis*. *Curr. Biol.* **20**, 951–956.

Law J. A., Vashisht A. A., Wohlschlegel J. A. and Jacobsen S. E. 2011 SHH1, a homeodomain protein required for DNA methylation, as well as RDR2, RDM4, and chromatin remodeling factors, associate with RNA polymerase IV. *PLoS Genet.* **7**, e1002195.

Li C. F., Pontes O., El-Shami M., Henderson I. R., Bernatavichute Y. V., Chan S. W.-L. *et al.* 2006 An ARGONAUTE4-containing nuclear processing center colocalized with Cajal bodies in *Arabidopsis thaliana*. *Cell* **126**, 93–106.

Lindroth A. M., Cao X., Jackson J. P., Zilberman D., McCallum C. M., Henikoff S. and Jacobsen S. E. 2001 Requirement of *CHROMOMETHYLASE 3* for maintenance of CpXpG methylation. *Science* **202**, 2077–2080.

Lindroth A. M., Shultis D., Jasencakova Z., Fuchs J., Johnson L., Schubert D. *et al.* 2004 Dual histone H3 methylation marks at lysine 9 and 27 required for interaction with *CHROMOMETHYLASE 3*. *EMBO J.* **23**, 4286–4296.

Lippman Z., Gendrel A. V., Black M., Vaughn M. W., Dedhia N., McCombie W. R. *et al.* 2004 Role of transposable elements in heterochromatin and epigenetic control. *Nature* **430**, 471–476.

Lira-Medeiros C. F., Parisod C., Fernandes R. A., Mata C. S., Cardosa M. A. and Ferreira P. C. 2010 Epigenetic variation in mangrove plants occurring in contrasting natural environment. *PLoS ONE* **5**, e10326.

Lisch D. 2009 Epigenetic regulation of transposable elements in plants. *Ann. Rev. Plant Biol.* **60**, 43–66.

Lisch D. and Bennetzen J. L. 2011 Transposable element origins of epigenetic regulation. *Curr. Opin. Plant Biol.* **14**, 156–161.

Lister R., O'Malley R. C., Tonti-Filippni J., Gregory B. D., Berry C. C., Millar A. H. and Ecker J. R. 2008 Highly integrated single-base resolution maps of the epigenome in *Arabidopsis*. *Cell* **133**, 523–536.

Lu C., Kulkarni K., Souret F. F., MuthuValliappan R., Tej S. S., Poethig R. S. *et al.* 2006 MicroRNAs and other small RNAs enriched in the *Arabidopsis* RNA-dependent RNA Polymerase-2 mutant. *Genome Res.* **16**, 1276–1288.

Lu Y., Rong T. and Cao M. 2008 Analysis of DNA methylation in different maize tissues. *J. Genet. Genomics* **35**, 41–48.

Lucht J. M., Mauch-Mani B., Steiner H. Y., Metraux J. P., Ryals J. and Hohn B. 2002 Pathogen stress increases somatic recombination frequency in *Arabidopsis*. *Nat. Genet.* **30**, 311–314.

Luff B., Pawlowski L. and Bender J. 1999 An inverted repeat triggers cytosine methylation of identical sequences in *Arabidopsis*. *Mol. Cell* **3**, 505–511.

Luna E., Bruce T. J., Roberts M. R., Flors V. and Ton J. 2012 Next-generation systemic acquired resistance. *Plant Physiol.* **158**, 844–853.

Luo C. and Lam E. 2010 ANCORP: a high-resolution approach that generates distinct chromatin state models from multiple genome-wide datasets. *Plant J.* **63**, 339–351.

Luo M., Taylor J. M., Spriggs A., Zhang H., Wu X., Russell S. *et al.* 2011 A genome-wide survey of imprinted genes in rice seeds reveals imprinting primarily occurs in the endosperm. *PLoS Genet.* **7**, e1002125.

Lyko F., Ramsahoye B. H. and Jaenisch R. 2000 DNA methylation in *Drosophila melanogaster*. *Nature* **408**, 538–546.

Ma J., Wing R. A., Bennetzen J. L. and Jackson S. A. 2007 Plant centromere organization: a dynamic structure with conserved functions. *Trends Genet.* **23**, 134–139.

Maloisel L. and Rossignol J. L. 1998 Suppression of crossing-over by DNA methylation in *Ascobolus*. *Genes Dev.* **12**, 1381–1389.

Manning K., Tor M., Poole M., Hong Y., Thompson A. J., King G. J. *et al.* 2006 A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat. Genet.* **38**, 948–952.

March-Diaz R., Garcia-Dominquez M., Lozano-Juste J., Leon J., Florencio F. J. and Reyes J. C. 2008 Histone H2A.Z and homologues of components of *SWR1* complex are required to control immunity in *Arabidopsis*. *Plant J.* **53**, 475–487.

Margueron R. and Reinberg D. 2011 The polycomb complex PRC2 and its mark in life. *Nature* **469**, 343–349.

Morris C. A. and Moazed D. 2007 Centromere assembly and propagation. *Cell* **128**, 647–650.

Martin A., Troadec C., Boualem A., Rajab M., Fernandez R., Morin H. *et al.* 2009 A transcription-induced epigenetic change leads to sex determination in melon. *Nature* **461**, 1135–1138.

Martinez-Macias M. I., Quin W., Miki D., Pontes O., Liu Y., Tang K. *et al.* 2012 A DNA 3′ phosphatase functions in active DNA demethylation in *Arabidopsis*. *Mol. Cell* **45**, 357–370.

Matsunaga W., Kobayashi A., Kato A. and Ito H. 2012 The effects of heat induction and the siRNA biogenesis pathway on the transgenerational transcription of ONSEN, a copia-like retrotransposon in *Arabidopsis thaliana*. *Plant Cell Physiol.* **53**, 824–833.

Matzke M., Kanno T., Huettel B., Daxinger L. and Matzke A. J. 2007 Targets of RNA-directed DNA methylation. *Curr. Opin. Plant Biol.* **10**, 512–519.

Maunakea A. K., Nagarajan R. P., Bilenky M., Ballinger T. J., D'Souza C., Fouse S. D. *et al.* 2010 Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* **466**, 253–257.

Maury S., Trap-Gentil M. V., Hebrard C., Weyens G., Delaunay A., Barnes S. *et al.* 2012 Genic DNA methylation changes during *in vitro* organogenesis: organ specificity and conservation between parental lines of epialleles. *Physiol. Plant* **146**, 321–325.

Mayr E. 1984 What is Darwinism today? *Proceedings of the Biennial Meeting of the Philosophy of Science Association*. Vol. 2. Symposia and Invited Papers, pp. 145–156, University of Chicago Press, Chicago, USA.

Melquist S., Luff B. and Bender J. 1999 *Arabidopsis PAI* gene arrangements, cytosine methylation and expression. *Genetics* **153**, 401–413.

Messeguer R., Ganal M. W., Steffens J. C. and Tanksley S. D. 1991 Characterization of the level, target sites and inheritance of cytosine methylation in tomato nuclear DNA. *Plant Mol. Biol.* **16**, 753–770.

Mi S., Cai T., Hu Y., Chen Y., Hodges E., Ni F. *et al.* 2008 Sorting of small RNAs into *Arabidopsis* argonaute complex is directed by the 5′ terminal nucleoide. *Cell* **133**, 116–127.

Mirouze M., Lieberman-Lazarovich M., Aversano R., Bucher E., Nicolet J., Reinders J. and Paszkowski J. 2012 Loss of DNA methylation affects the recombination landscape in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **109**, 5880–5885.

Mirouze M., Reinders J., Bucher E., Nishimura T., Schneeberger K., Ossowski S. *et al.* 2009 Selective epigenetic control of retrotransposition in *Arabidopsis*. *Nature* **461**, 427–430.

Miura A., Yonebayashi S., Watanabe K., Toyama T., Shimada H. and Kakutani T. 2001 Mobilization of transposons by a mutation abolishing full DNA methylation in *Arabidopsis*. *Nature* **411**, 212–214.

Miura A., Nakamura M., Inagaki S., Kobayashi A., Saze H. and Kakutani T. 2009 An *Arabidopsis* jmjC domain protein protects transcribed genes from DNA methylation at CHG sites. *EMBO J.* **28**, 1078–1086.

Mizuguchi G., Shen X., Landry J., Wu W. H., Sen S. and Wu C. 2004 ATP-driven exchange of histone H2A.Z variant catalyzed by SWR1 chromatin remodelling complex. *Science* **303**, 343–348.

Mlynarova L., Nap J. P. and Bisseling T. 2007 The SWI/SNF chromatin-remodeling gene AtCHR12 mediates temporary growth arrest in *Arabidopsis thaliana* upon perceiving environmental stress. *Plant J.* **51**, 874–885.

Molinier J., Ries G., Zipfel C. and Hohn B. 2006 Transgeneration memory of stress in plants. *Nature* **442**, 1046–1049.

Morales-Ruiz T., Ortega-Galisteo A. P., Ponferrada-Marin M. I., Martinez-Macias M. I., Ariza R. R. and Roldan-Arjona T. 2006 *DEMETER* and *REPRESSOR OF SILENCING 1* encode 5-methylcytosine DNA glycosylases. *Proc. Natl. Acad. Sci. USA* **103**, 6853–6858.

Nagaki K., Talbert P. B., Zhong C. X., Dawe R. K., Henikoff S. and Jiang J. 2003 Chromatin immunoprecipitation reveals that the 180-bp satellite repeat is the key functional DNA element of *Arabidopsis thaliana* centromeres. *Genetics* **163**, 1221–1225.

Naito K., Zhang F., Tsukiyama T., Saito H., Hancock C. N., Richardson A. O. *et al.* 2009 Unexpected consequences of a sudden and massive transposon amplification on rice gene expression. *Nature* **461**, 1130–1134.

Nakano Y., Steward N., Sekine M., Kusano T. and Sano H. 2000 A tobacco *NtMET1* cDNA encoding a DNA methyltransferase: molecular characterization and abnormal phenotypes of transgenic tobacco plants. *Plant Cell Physiol.* **41**, 448–457.

Ng H. H., Jeppesen P. and Bird A. 2000 Active repression of methylated genes by the chromosomal protein MBD1. *Mol. Cell Biol.* **20**, 1394–1406.

Nosaka M., Itoh J., Nagato Y., Ono A., Ishiwata A. and Sato Y. 2012 Role of transposon-derived small RNAs in the interplay between genomes and parasitic DNA in rice. *PLoS Genet.* **9**, e1002953.

Noutoshi Y., Ikeda M., Saito T., Osada H. and Shirasu K. 2012a Sulfonamides identified as plant-immune priming compounds in high-throughput chemical screening increase disease resistance in *Arabidopsis thaliana*. *Front. Plant Sci.* **3**, 245.

Noutoshi Y., Ikeda M. and Shirasu K. 2012b Diuretics prime plant immunity in *Arabidopsis thaliana*. *PLoS ONE* **7**, e48443.

Noutoshi Y., Okazaki M., Kida T., Nishina Y., Morishita Y., Ogawa T. *et al.* 2012c Novel plant immune-priming compounds identified via high-throughput chemical screening target salicylic glucosyl transferase in *Arabidopsis*. *Plant Cell* **24**, 3795–3804.

Olmedo-Monfil V., Duran-Figueroa N., Arteaga-Vazquez M., Demesa-Arevalo E., Autran D., Grimanelli D. *et al.* 2010 Control of female gamete formation by a small RNA pathway in *Arabidopsis*. *Nature* **464**, 628–632.

Onodera Y., Haag J. R., Ream T., Nunes P. C., Pontes O. and Pikaard C. S. 2005 Plant nuclear RNA polymerase IV mediates siRNA and DNA methylation-dependent heterochromatin formation. *Cell* **120**, 613–622.

Ortega-Galisteo A. P., Morales-Ruiz T., Ariza R. R. and Roldan-Arjona T. 2008 *Arabidopsis DEMETER-LIKE* proteins DML2 and DML3 are required for appropriate distribution of DNA methylation marks. *Plant Mol. Biol.* **67**, 671–681.

Ossowski S., Schneeberger K., Lucas-Lledo J. I., Warthmann N., Clark R. M., Shaw R. G. *et al.* 2010 The rate and molecular spectrum of spontaneous mutation in *Arabidopsis thaliana*. *Science* **327**, 92–94.

Ou X., Zhang Y., Xu C., Lin X., Zang Q., Zhuang T. *et al.* 2012 Transgenerational inheritance of modified DNA methylation patterns and enhanced tolerance induced by heavy metal stress in rice (*Oryza sativa* L.). *PLoS ONE* **7**, e41143.

Papamichos-Chronakis M., Watanabe S., Rando O. J. and Peterson C. L. 2011 Global regulation of H2A.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity. *Cell* **144**, 200–213.

Paun O., Bateman R. M., Fay M. F., Hedren M., Civeyrel L. and Chase M. W. 2010 Stable epigenetic effects impact adaptation in allopolyploid orchids (Dactylorhiza: Orchidaceae). *Mol. Biol. Evol.* **27**, 2465–2473.

Pavet V., Quintero C., Cecchini N. M., Rosa A. L. and Alvarez M. E. 2006 *Arabidopsis* displays centromeric DNA hypomethylation and cytological aleterations of heterochromatin upon attack by *Pseudomonas syringae*. *MPMI* **19**, 577–587.

Penterman J., Zilberman D., Huh J. H., Ballinger T., Henikoff S. and Fischer R. L. 2007 DNA demethylation in the *Arabidopsis* genome. *Proc. Natl. Acad. Sci. USA* **104**, 6752–6757.

Pfeifer G. P. 2006 Mutagenesis at methylated CpG sequences. *CTMI* **301**, 259–281.

Pick T., Jaskiewicz M., Peterhansel C. and Conrath U. 2012 Heat shock factor HsfB1 primes gene transcription and systemic acquired resistance in *Arabidopsis*. *Plant Physiol.* **159**, 52–55.

Piegu B., Guyot R., Picault N., Roulin A., Sanyal A., Kim H. *et al.* 2006 Doublimg genome size without polyploidization: dynamics of retrotransposition-driven genomic expansions in *Oryza australiensis*, a wild relative of rice. *Genome res.* **16**, 1262–1269.

Pikaard C. S., Haag J. R., Ream T. and Wierzbicki A. T. 2008 Roles of RNA polymerase IV in gene silencing. *Trends Plant Sci.* **13**, 390–397.

Ponferrada-Marin M. I., Roldan-Arjona T. and Ariza R. R. 2012 Demethylation initiated by ROS1 glycosylase involves random sliding along DNA. *Nucleic Acids Res.* **40**, 11554–11562.

Pontes O., Li C. F., Costa Nunes P., Haag J., Ream T., Vitins A. *et al.* 2006 The *Arabidopsis* chromatin-modifying nuclear siRNA pathway involves a nucleolar RNA processing center. *Cell* **14**, 79–92.

Pontvianne F., Blevins T. and Pikaard C. S. 2010 *Arabidopsis* histone lysine methyltransferases. *Adv. Bot. Res.* **53**, 1–22.

Probst A. V., Fagard M., Proux F., Mourrain P., Boutet S., Earley K. *et al.* 2004 *Arabidopsis* histone deacetylase HDA6 is required for maintenance of transcriptional gene and determines nuclear organization of rDNA repeats. *Plant Cell* **16**, 1021–1034.

Qi Y., He X., Wang X-J., Kohany O., Jurka J. and Hannon G. J. 2006 Distinct catalytic and non-catalytic roles of ARGONAUTE 4 in RNA-directed DNA methylation. *Nature* **443**, 1006–1012.

Rabinowicz P. D., Palmer L. E., May B. P., Hemann M. T., Lowe S. W., McCombie W. R. and Martienssen R. A. 2003 Genes and transposons are differentially methylated in plants, but not in mammals. *Genome Res.* **13**, 2658–2664.

Raissig M. T., Baroux C. and Grossniklaus U. 2011 Regulation and flexibility of genomic imprinting during seed development. *Plant Cell* **23**, 16–26.

Rangwala S. H. and Richards E. J. 2007 Differential epigenetic regulation within an *Arabidopsis* retrotransposon family. *Genetics* **176**, 151–160.

Rasmann S., De Vos M., Casteel C. L., Tian D., Halitschke R., Sun J. Y. *et al.* 2012 Herbivory in the previous generation primes plants for enhanced insect resistance. *Plant Physiol.* **158**, 854–863.

Ream T. S., Haag J. R., Wierzbicki A. T., Nicora C. D., Norbeck A. D., Zhu J. K. *et al.* 2009 Subunit compositions of the RNA-silencing enzymes Pol IV and Pol V reveal their origins as specialized forms of RNA polymerase II. *Mol. Cell* **33**, 192–203.

Reinders J., Wulff B. B., Mirouze M., Mari-Ordonez A., Dapp M., Rozhon W. *et al.* 2009 Compromised stability of DNA methylation and transposon immobilization in mosaic *Arabidopsis* epigenomes. *Genes Dev.* **23**, 939–950.

Reyes J. C. 2006 Chromatin modifiers that control plant development. *Curr. Opin. Plant Biol.* **9**, 21–27.

Rival A., Jaligot E., Beule T. and Finnegan E. J. 2008 Isolation and expression analysis of genes encoding MET, CMT and DRM methyltransferses in oil palm (*Elaeis guineensis* Jacq.) in relation to the 'mantled' somaclonal variation. *J. Exp. Bot.* **59**, 3271–3281.

Roudier F., Ahmed I., Berard C., Sarazin A., Mary-Huard T., Cortijo S. *et al.* 2011 Integrative epigenomic mapping defines four main chromatin states in *Arabidopsis*. *EMBO J.* **30**, 1928–1938.

Ruiz-Garcia L., Cervera M. T. and Martinez-Zapater J. M. 2005 DNA methylation increases throughtout *Arabidopsis* development. *Planta* **222**, 301–306.

Sano H., Kamada I., Youssefian S., Katsumi M. and Wabilko H. 1990 A single treatment of rice seedlings with 5-azacytidine induces heritable dwarfism and undermethylation of genomic DNA. *Mol. Gen. Genet.* **220**, 441–447.

Sasai N. and Defossez P. A. 2009 Many paths to one goal? The proteins that recognize methylated DNA in eukaryotes. *Int. J. Dev. Biol.* **53**, 323–334.

Sasaki T., Kobayashi A., Saze H. and Kakutani T. 2012a RNAi-independent *de novo* DNA methylation revealed in *Arabidopsis* mutants of chromatin remodeling gene DDM1. *Plant J.* **70**, 750–758.

Sasaki T., Naumann U., Forai P., Matzke A. J. and Matzke M. 2012b Unusual case of apparent hypermutation in *Arabidopsis thaliana*. *Genetics* **192**, 1271–1280.

Saze H. and Kakutani T. 2007 Heritable epigenetic mutation of a transposon-flanked *Arabidopsis* gene due to lack of the chromatin-remodeling factor DDM1. *EMBO J.* **26**, 3641–3652.

Saze H. and Kakutani T. 2011 Differentiation of epigenetic modifications between transposons and genes. *Curr. Opin. Plant Biol.* **14**, 81–87.

Saze H., Shiraishi A., Miura A. and Kakutani T. 2008 Control of genic DNA methylation by a jmjC domain-containing protein in *Arabidopsis thaliana*. *Science* **319**, 462–465.

Saze H., Tsugane K., Kanno T. and Nishimura T. 2012 DNA methylation in plants: relationship to small RNAs and histone modifications and functions in transposon inactivation. *Plant Cell Physiol.* **53**, 766–784.

Scebba F., Bernacchia G., De Bastiani M., Evangelista M., Cantoni R. M., Cella R. *et al.* 2003 *Arabidopsis* MBD proteins show different binding specificities and nuclear localization. *Plant Mol. Biol.* **53**, 715–731.

Schmitz R. J., Schultz M. D., Lawsey M. G., O'Malley R. C., Urich M. A., Libiger O. *et al.* 2011 Transgenerational epigenetic instability is a source of novel methylation variants. *Science* **334**, 369–373.

Scoville A. G., Barnett L. L., Bodbyl-Roels S., Kelly J. K. and Hileman L. C. 2011 Differential regulation of a MYB transcription factor is correlated with transgenerational epigenetic inheritance of trichome density in *Mimulus guttatus*. *New Phytol.* **191**, 251–263.

Searle I. R., Pontes O., Melnyk C. W., Smith L. M. and Baulcombe D. C. 2010 JMJ14; a JmjC domain protein, is required for RNA silencing and cell-to-cell movement of an RNA silencing signal in *Arabidopsis*. *Genes Dev.* **24**, 986–991.

Sha A. H., Lin X. H., Huang J. B. and Zhang D. P. 2005 Analysis of DNA methylation related to rice adult plant resistance to bacterial blight based on methylation-sensitive AFLP (MSAP) analysis. *Mol. Genet. Genomics* **273**, 484–490.

Shaked H., Kashkush K., Ozkan H., Feldman M. and Levy A. A. 2001 Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *Plant Cell* **13**, 1749–1759.

Shiba H., Kakizaki T., Iwano M., Tarutani Y., Watanabe M., Isogai A. and Takayama S. 2006 Dominance relationships between self-incompatibility alleles controlled by DNA methylation. *Nat. Genet.* **38**, 297–299.

Singh M., Goel S., Meeley R. B., Dantec C., Parrinello H., Michaud C. *et al.* 2011 Production of variable gametes without meiosis in maize deficient for an ARGONAUTE protein. *Plant Cell* **23**, 443–458.

Slaughter A., Daniel X., Flors V., Luna E., Hohn B. and Mauch-Mani B. 2012 Descendants of primed *Arabidopsis* plants exhibit resistance to biotic stress. *Plant Physiol.* **158**, 835–843.

Slotkin R. K., Freeling M. and Lisch D. 2005 Heritable transposon silencing initiated by a naturally occurring transposon inverted duplication. *Nat. Genet.* **37**, 641–644.

Slotkin R. K., Vaughn M., Borges F., Tanurdzic M., Becker J. D., Feijo J. A. and Martienssen R. A. 2009 Epigenetic reprogramming and small RNA silencing of transposable elements in pollen. *Cell* **136**, 461–472.

Smith L. M., Pontes O., Searle I., Yelina N., Yousafzai F. K., Herr A. J. *et al.* 2007 An SNF2 protein associated with nuclear RNA silencing and the spread of a silencing signal between cells in *Arabidopsis*. *Plant Cell* **19**, 1507–1521.

Song Y., Ji D., Li S., Wang P., Li Q. and Xiang F. 2012 The dynamic changes of DNA methylation and histone modifications of salt responsive transcription factor genes in soybean. *PLoS ONE* **7**, e41274.

Stam M., Belele C., Dorweiler J. E. and Chandler V. L. 2002 Differential chromatin structure within tandem array 100 kb upstream of the maize b1 locus is associated with paramutation. *Genes Dev.* **16**, 1906–1918.

Steward N., Kusano T. and Sano H. 2000 Expression of *ZmMET1*, a gene encoding a DNA methyltransferase from maize, is associated not only with DNA replication in actively proliferating cells, but also with altered DNA methylation status in cold-stressed quiescent cells. *Nucleic Acids Res.* **28**, 3250–3259.

Steward N., Ito M., Yamaguchi Y., Koizumi N. and Sano H. 2002 Periodic DNA methylation in maize nucleosomes and demethylation by environmental stress. *J. Biol. Chem.* **277**, 37741–37746.

Stonaker J. L., Lim J. P., Erhard Jr K. F. and Hollick J. B. 2009 Diversity of Pol IV function is defined by mutations at the maize *rmr7* locus. *PLoS Genet.* **5**, e1000706.

Tarutani Y., Shiba H., Iwano M., Tarutani Y., Watanabe M., Isogai A. and Takayama S. 2010 Trans-acting small RNA determines dominance relationships in Brassica self-incompatibilty. *Nature* **466**, 983–986.

Teixeira F. K., Heredia F., Sarazin A., Roudier F., Boccara M., Ciaudo C. *et al.* 2009 A role for RNAi in the selective correction of DNA methylation defects. *Science* **323**, 1600–1604.

Tittel-Elmer M., Bucher E., Broger L., Mathieu O., Paszkowski J. and Valliank J. 2010 Stress-induced activation of heterochromatic transcription. *PLoS ONE* **6**, e1001175.

Tiwari S., Schulz R., Ikeda Y., Dytham L., Bravo J., Mathers L. *et al.* 2008 *MATERNALLY EXPRESSED PAB C-TERMINAL*., a novel imprinted gene in *Arabidopsis*, encodes the conserved C-terminal domain of polyadenylate binding proteins. *Plant Cell* **20**, 2387–2398.

To T. K., Kim J. M., Matsui A., Kurihara Y., Morosawa T., Ishida J. *et al.* 2011a *Arabidopsis* HDA6 regulated locus-directed heterochromatin silencing in cooperation with MET1. *PLoS Genet* **7**, e1002055.

To T. K., Nakaminami K., Kim J. M., Morosawa T., Ishida J., Tanaka M. *et al.* 2011b *Arabidopsis* HDA6 is required for freezing tolerance. *Biochem. Biophys. Res. Commun.* **406**, 414–419.

Topp C. N. and Dawe R. K. 2006 Reinterpreting pericentromeric heterochromatin. *Curr. Opin. Plant Biol.* **9**, 647–653.

Tsukahara S., Kobayashi A., Kawabe A., Mathieu O., Miura A. and Kakutani T. 2009 Bursts of retrotransposition reproduced in *Arabidopsis*. *Nature* **461**, 423–426.

Vanyushin B. F. 2006 DNA methylation in plants. *Curr. Topics Microbiol. Immunol.* **301**, 67–122.

Vaughn M. W., Tanurdzic M., Lippman Z., Jiang H., Carrasquillo R., Rabinowicz P. D. *et al.* 2007 Epigenetic natural variation in *Arabidopsis thaliana*. *PLoS Biol.* **5**, e174.

Verhoeven K. J., Jansen J. J., van Dijk P. J. and Biere A. 2010 Stress-induced DNA methylation changes and their heritability in asexual dandelions. *New Phytol.* **185**, 1108–1118.

Vielle-Calzada J. P., Thomas J., Spillane C., Coluccio A., Hoeppner M. A. and Grossniklaus U. 1999 Maintenance of genomic imprinting at the *Arabidopsis medea* locus requires zygotic DDM1 activity. *Genes Dev.* **13**, 2971–2982.

Vitte C. and Bennetzen J. L. 2006 Analysis of retrotransposon structural diversity uncovers properties and propensities in angiosperm genome evolution. *Proc. Natl. Acad. Sci. USA* **103**, 17638–17643.

Vogel F. and Schroeder T. M. 1974 The internal order of the interphase nucleus. *Humangenetik* **25**, 265–297.

Wada Y., Miyamoto K., Kusano H. and Sano H. 2004 Association between up-regulation of stress-responsive genes and hypomethylation of genomic DNA in tobacco plants. *Mol. Genet. Genomics* **271**, 658–666.

Walsh C. P. and Xu G. L. 2006 Cytosine methylation and DNA repair. *Curr. Top Microbiol. Immunol.* **301**, 283–315.

Wang W. S., Pan Y. J., Zhao X. Q., Dwivedi D., Zhu L. H., Ali J. *et al.* 2011 Drought-induced site-specific DNA methylation and its association with drought tolerance in rice (*Oryza sativa* L.). *J. Exp. Bot.* **62**, 1951–1960.

Wassenegger M. 2005 The role of the RNAi machinery in heterochromatin formation. *Cell* **122**, 13–16.

Wessler S. R. 1996 Turned on by stress. Plant retrotransposons. *Curr. Biol.* **6**, 959–961.

Wierzbicki A. T., Haag J. R. and Pikaard C. S. 2008 Non-coding transcription by RNA polymerase Pol IVb/Pol V mediates transcriptional silencing of overlapping and adjacent genes. *Cell* **135**, 635–648.

Wierzbicki A. T., Ream T. S., Haag J. R. and Pikaard C. S. 2009 RNA polymerase V transcription guides *ARGONAUTE4* to chromatin. *Nat. Genet.* **41**, 630–634.

Wierzbicki A. T., Cocklin R., Mayampurath A., Lister R., Rowley M. J., Gregory B. D. *et al.* 2012 Spatial and functional relationships among Pol V-associated loci, Pol IV-dependent siRNAs, and cytosine methylation in the *Arabidopsis* epigenome. *Genes Dev.* **26**, 1825–1836.

Woo H. R. and Richards E. 2008 Natural variation in DNA methylation in ribosomal RNA genes of *Arabidopsis thaliana*. *BMC Plant Biol.* **8**, 92.

Woo H. R., Pontes O., Pikaard C. S. and Richards E. J. 2007 VIM1, a methylcytosine-binding protein required for centromeric heterochromatization. *Genes Dev.* **21**, 267–277.

Wu S. C. and Zhang Y. 2010 Active DNA demethylations: many roads lead to Rome. *Nat. Rev. Mol. Cell Biol.* **11**, 607–620.

Wu W. H., Alami S., Luk K., Wu C. H., Sen S., Mizuguchi G. *et al.* 2005 Swc2 is a widely conserved H2AZ-binding module essential for ATP-dependent histone exchange. *Nat. Struct. Mol. Biol.* **12**, 1064–1071.

Wu K., Zhang L., Zhou C., Yu C. W. and Chaikam V. 2008 *HDA6* is required for jasmonate response, senescence and flowering in *Arabidopsis*. *J. Exp. Biol.* **59**, 225–234.

Xiao W. 2012 Specialized technologies for epigenetics in plants. *Methods Mol. Biol.* **925**, 231–247.

Xiao W., Custard K. D., Brown R. C., Lemmon B. E., Harada J. J., Goldberg R. B. and Fischer R. L. 2006 DNA methylation is critical for *Arabidopsis* embryogenesis and seed viability. *Plant Cell* **18**, 805–814.

Xie Z., Johansen L. K., Gustafson A. M., Kasschau K. D., Lellis A. D., Zilberman D. *et al.* 2004 Genetic and functional diversification of small RNA pathways in plants. *PLoS Biol.* **2**, e104.

Xiong L. Z., Xu C. G., Saghai Maroof M. A. and Zhang Q. 1999 Patterns of cytosine methylation in an elite rice hybrid

and its parental lines, detected by a methylation-sensitive amplification polymorphism technique. *Mol. Gen. Genet.* **261**, 439–446.

Yang Z., Ebright Y. W., Yu B. and Chen X. 2006 HEN1 recognizes 21–24 nt small RNA duplexes and deposits a methyl group on to the 2′OH of the 3′ terminal nucleotide. *Nucleic Acids Res.* **34**, 667–675.

Yao Y. and Kovalchuk I. 2011 Abiotic stress leads to somatic and heritable changes in homologous recombination frequency, point mutation frequency and microsatellite stability in *Arabidopsis* plants. *Mut. Res.* **707**, 61–66.

You W., Tyczewska A., Spencer M., Daxinger L., Schmid M. W., Grossniklaus U. *et al.* 2012 Atypical DNA methylation of genes encoding cysteine-rich peptides in *Arabidopsis thaliana*. *BMC Plant Biol.* **12**, 51–65.

Yu B., Yang Z., Li J., Minakhina S., Yang M., Padgett R. W., Steward R. and Chen X. 2005 Methylation as a crucial step in plant microRNA biogenesis. *Science* **307**, 932–935.

Yu A., Lepere G., Jay F., Wang J., Bapaume L., Wang Y. *et al.* 2013 Dynamics and biological relevance of DNA demethylation in *Arabidopsis* antibacterial defense. *PNAS* **110**, 2389–2394.

Zemach A. and Grafi G. 2003 Characterization of *Arabidopsis thaliana* methyl-CpG-binding domain proteins. *Plant J.* **34**, 565–572.

Zemach A. and Grafi G. 2007 Methyl-CpG-binding domain protein in plants: interpreters of DNA methylation. *Trends Plant Sci.* **12**, 80–85.

Zemach A., Li Y., Wayburn B., Ben-Meir H., Kiss V., Avivi Y. *et al.* 2005 DDM1 binds *Arabidopsis* methyl-CpG binding domain proteins and affects their subnuclear localization. *Plant Cell* **17**, 1549–1558.

Zemach A., McDaniel I. E., Silva P. and Zilberman D. 2010 Genome-wide evolutionary analysis of eukaryotic DNA methylation. *Science* **328**, 916–919.

Zhang X. 2008 The epigenetic landscape of plants. *Science* **320**, 489–492.

Zhang X., Henderson I. R., Lu C., Green P. J. and Jacobsen S. E. 2007 Role of RNA polymerase IV in plant small RNA metabolism. *Proc. Natl. Acad. Sci. USA* **104**, 4536–4541.

Zhang X., Bernatavichute Y. V., Cokus S., Pellegrini M. and Jacobsen S. E. 2009 Genome-wide analysis of mono-, di- and tri-methylation of histone H3 lysine 4 in *Arabidopsis thaliana*. *Genome Biol.* **10**, R62.

Zhang W., Friebe B., Gill B. S. and Jiang J. 2010 Centromere inactivation and epigenetic modifications of a plant chromosome with three functional centromeres. *Chromosoma* **119**, 553–563.

Zhang M., Zhao H., Xie S., Chen J., Xu Y., Wang K. *et al.* 2011 Extensive, clustered parental imprinting of protein-coding and noncoding RNAs in developing maize endosperm. *Proc. Natl. Acad. Sci. USA* **108**, 20042–20047.

Zhao Y., Yu S., Xing C., Fan S. and Song M. 2008 DNA methylation in cotton hybrids and their parents. *Mol. Biol.* **42**, 169–178.

Zhao Y., Yu S., Ye W., Wang H., Wang J. and Fang B. 2010 Study on DNA cytosine methylation of cotton (*Gossypium hirsutum* L.) genome and its implications for salt tolerance. *Agri. Sci. China* **9**, 783–791.

Zheng B. and Chen X. 2011 Dynamics of histone H3 lysine 27 trimethylation in plant development. *Curr. Opin. Plant Biol.* **14**, 123–129.

Zheng X., Zhu J., Kapoor A. and Zhu J. K. 2007 Roles of *Arabidopsis* AGO6 in siRNA accumulation, DNA methylation and transcriptional gene silencing. *EMBO J.* **26**, 1691–1701.

Zheng X., Pontes O., Zhu J., Miki D., Zhang F., Li W. X. *et al.* 2008 ROS3 is an RNA-binding protein required for DNA demethylation in *Arabidopsis*. *Nature* **455**, 1259–1262.

Zheng B., Wang Z., Li S., Yu B., Liu J. Y. and Chen X. 2009 Intergenic transcription by RNA polymerase II coordinates pol IV and pol V in siRNA-directed transcriptional gene silencing in *Arabidopsis*. *Genes Dev.* **23**, 2850–2860.

Zheng Z., Xing Y., He X. J., Li W., Hu Y., Yadav S. K. *et al.* 2010 An SGS3-like protein functions in RNA-directed DNA methylation and transcriptional gene silencing in *Arabidopsis*. *Plant J.* **62**, 92–99.

Zhong L., Xu Y.-H. and Wang J.-B. 2009 DNA-methylation changes induced by salt stress in wheat, *Triticum aestivum*. *Afr. J. Biotech.* **8**, 6201–6207.

Zhu J. K. 2009 Active DNA methylation mediated by DNA glycosylases. *Ann. Rev. Genet.* **43**, 143–166.

Zhu J., Kapoor A., Sridhar V. V., Agius F. and Zhu J. K. 2007 The DNA glycosylase/lyase ROS1 functions in pruning DNA methylation patterns in *Arabidopsis*. *Curr. Biol.* **17**, 54–59.

Zhu R., Shevchenko O., Ma C., Maury S., Freitag M. and Strauss S. H. 2013 Poplars with a *PtDDM-RNAi* transgene have reduced DNA methylation and show aberrant post-dormancy morphology. *Planta* **237**, 1483–1493.

Zilberman D., Cao X. and Jacobsen S. E. 2003 *ARGONAUTE 4* control of locus-specific siRNA accumulation and DNA histone methylation. *Science* **299**, 716–719.

Zilberman D., Cao X., Johansen L. K., Xie Z., Carrington J. C. and Jacobsen S. E. 2004 Role of *Arabidopsis ARGONAUTE4* in RNA-directed DNA methylation triggered by inverted repeats. *Curr. Biol.* **14**, 1214–1220.

Zilberman D., Coleman-Derr D., Ballinger T. and Henikoff S. 2008 Histone H2A.Z and DNA methylation are mutually antagonistic chromatin marks. *Nature* **456**, 125–129.

Zubko E., Gentry M., Kunova A. and Meyer P. 2012 *De novo* DNA methylation activity of METHYLTRANSFERASE 1 (MET1) partially restores body methylation in *Arabidopsis thaliana*. *Plant J.* **71**, 1029–1037.

Received 25 October 2012, in revised form 7 April 2013; accepted 14 May 2013
Published on the Web: 23 October 2013