Chapter

Involvement of Epigenetic Regulation in Plant Defence during Biotic Stress

Jasmine M. Shah and Joyous T. Joseph

Abstract

Plants being organisms that lack locomotion and vocabulary, they are not privileged to escape and communicate during unfavourable conditions of biotic/abiotic stresses, like their animal counterparts. Therefore, plants have evolved with higher adaptive skills that tune them during unfavourable conditions. In this context, regulation of gene expression plays a crucial role in controlling the cellular pathways required for survival during unfavourable conditions. This chapter is about the epigenetic regulation of plant defence during biotic stress. Researchers have taken various approaches to understand the epigenetic regulation of plant defences and these approaches are described here. Epigenetic regulation also has the potential to be inherited and this phenomenon has aided plants for better adaption. Such reports on transgenerational memory during biotic stress in plants are also compiled. A deeper understanding of epigenetic regulation of defence pathways during biotic stress, and identification of epigenetic marks on the genomes, can aid the development of crop improvement strategies. With the recent advancement in epigenome editing, it should become possible to develop epigenetically improvised plants, devoid of genetic modification.

Keywords: epigenetic regulation, epigenetic modification, biotic stress, plant defence, heritable epigenetic changes, methylation

1. Introduction

Regulation of gene expression is the ultimate criteria that decide the role of each player in all cellular pathways. Gene regulation occurs in the nucleus and cytoplasm at multiple levels such as chromatin conformation, transcription regulation, post-transcription regulation, regulation of translation, protein modification, and protein degradation. Regulation at transcription is one of the prominent mechanisms as it involves the so called 'switching-on and switching-off' of genes. Gene regulation is required for organisms not only for their routine growth and maintenance but also for survival during unfavourable conditions. In this context, epigenetic regulation is of much significance because it not only offers adaptive skills to the organisms under stress but also has the potential to be heritable, thereby contributing to transgenerational memory. Owing to lack of locomotion and vocabulary in plants, like their animal counter parts, it is imperative for them to survive in unfavourable conditions such as biotic and abiotic stress. Therefore, plants have evolved with an

elaborate mechanism of gene regulation, especially epigenetic regulation. Researchers have deduced gene functions by adopting various approaches and the key findings in epigenetic regulation and transgenerational memory in plants during biotic stress are described in this chapter.

2. Promoters, the switches for perceiving stress-induced communications

Promoters serve the function of a switch as they are the site for the binding of RNA polymerase and other transcription regulators. Promoters harbour *cis* regulatory sequences that can perceive information from regulatory factors in *trans*. This mechanism serves as an apt platform for perceiving signals of growth, development, and survival during unfavourable conditions of stress. In this context, promoters of plants play a significant role as they can support the organism to thrive unfavourable conditions, which they cannot escape owing to their sessile nature. Plants have developed an elaborate defence mechanism to survive during conditions of biotic stresses such as herbivory and disease. The defence mechanism comprise of pathways such as systemic acquired resistance (SAR) and induced systemic resistance (ISR), apart from structural adaptations and certain constitutively expressing defence proteins [1]. The secret for survival during vivid conditions of biotic stresses, which the plants are forced to undergo, lies in the regulation of gene expression involved in defence. Regulation at the switches, the gene promoters, mainly involves response to transcription factors (TFs) and epigenetic modifications, which tune the transcription by RNA polymerase. Special TFs belonging to the families such as bZIP (basic leucine zipper), MYB (myeloblastosis), WRKY, and NAC [NAM (No apical meristem), ATAF (*Arabidopsis* 69 transcription activation factor), and CUC (cup-shaped cotyledon)] are required to perceive biotic stresses [2]. Epigenetic modulators of the entire genome, including the promoters, involve the DNA and histones modifiers, and small non-coding RNAs. Change in nuclear architecture was also found to be involved in the regulation of promoters [3]. Epigenetic changes associated with other genomic regions such as introns and not the promoter were also known to be involved in gene regulation as they could result in alternative splicing.

3. Epigenetic modifications

Epigenetic changes refer to all modifications or influences on the chromatin, except for changes in the DNA sequence [4, 5]. These changes if occurred in promoters or other regulatory regions can result in altered gene expression, thereby contributing to phenotype plasticity. Multiple factors can lead to epigenetic changes (**Figure 1**). Nucleotides in DNA, especially cytosine, undergo methylation/demethylation. In addition to methylation, histones can undergo other chemical modifications such as acetylation, phosphorylation, ubiquitylation, and sumoylation. Non-coding RNAs are involved in altering chromatin organisation and/or methylation of chromatin [6]. Replacement of histones by histone variants such as H3.3, H2A.X, and H2A.Z also influence chromatin organisation and gene expression [7, 8].

4. Different approaches taken to understand epigenetic regulation of plant defences

Discoveries in various aspects of epigenetic regulation in plants provided a deeper perception of various pathways, including defence. Different approaches

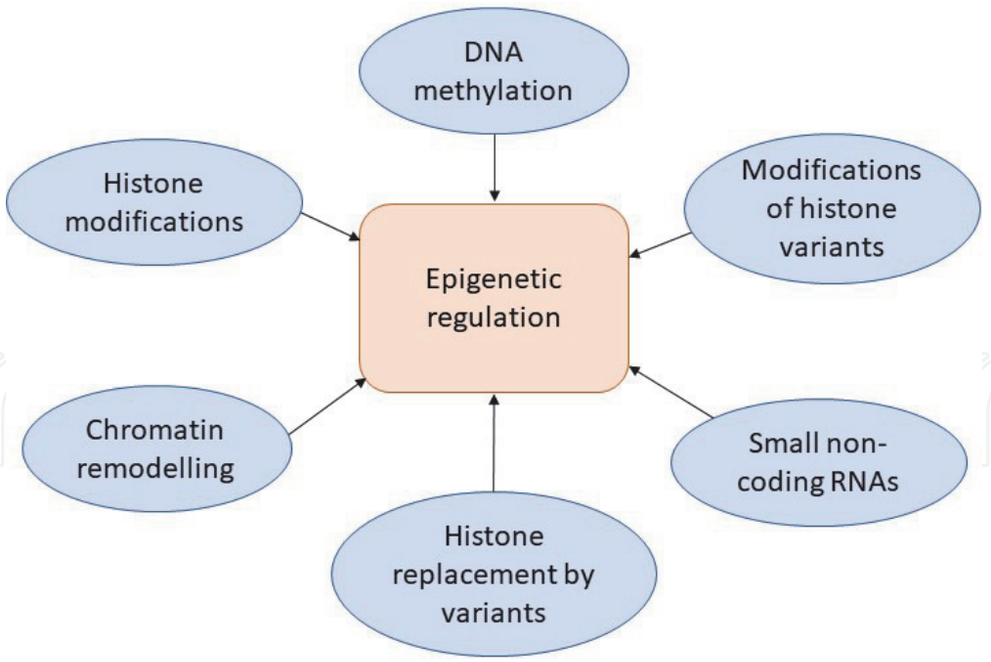

Figure 1.
Multiple causes of epigenetic regulation.

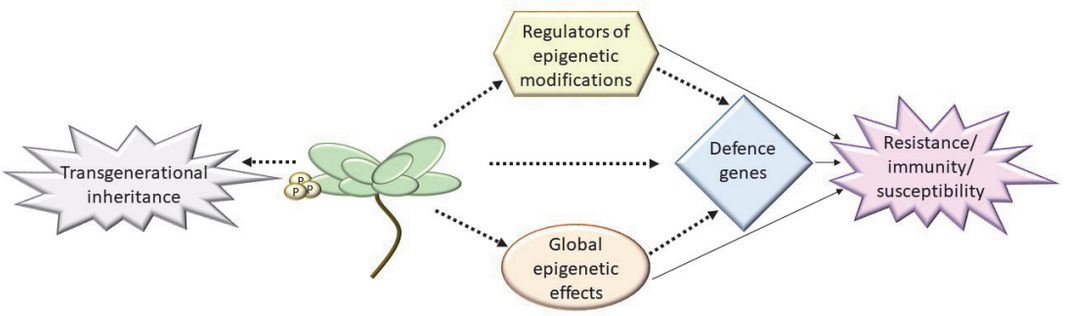

Figure 2.
Various paths chosen by researchers to understand the involvement of epigenetic regulation of plant defence, including the transgenerational memory of defence. The dotted arrows indicate the involvement of epigenetic modulations in that step.

taken by researchers include the study of epigenetic regulation of defence genes directly under pathogen stress, whole epigenome analysis, and understanding of the regulation of epigenetic regulators and their influence on disease (**Figure 2**). In this chapter, the research done in understanding different aspects of epigenetic regulation during plant-pathogen interaction and defence is described. Epigenetic changes have the potential to be retained after multiple cell divisions of both mitosis and meiosis. Therefore, heritable epigenetic changes lead to an interesting phenomenon of transgenerational memory. Epigenetic changes play a crucial role in plants as they not only lack locomotion, but also the vocabulary mode of communication. Biotic/abiotic stress-induced epigenetic changes have often generated plants and even their offsprings, with enhanced stress resistance. Such aspects are also elaborated in this chapter.

4.1 Biotic stress-induced epigenetic modulation of defence genes

Pathogen stress is well known to alter the expression of numerous genes, belonging to several pathways, including those involved in defence, in plants.

Interestingly, there are reports revealing the involvement of pathogen stress in altering the epigenetic status of various defence genes. For example, infection by *P. syringae* altered the histone methylation status of promoters of two key defence genes, *CPR5* and *PR1* [9]. Treatment with β-aminobutyric acid (BBA), which mimics infection, resulted in altered histone acetylation and methylation of promoters of few defence genes, *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1*), *ARABIDOPSIS NON-RACE SPECIFIC DISEASE RESISTANCE GENE (NDR1)/HAIRPIN-INDUCED GENE (HIN1)-LIKE 10* (*NHL10*) and *CYTOCHROME P450, FAMILY 81* (*CYP81F2*) and *PR1* of *Arabidopsis* [10]. This treatment also resulted in the priming of *Arabidopsis* plants against the bacterial pathogens *Pectobacterium carotovorum* ssp. *carotovorum* (*Pcc*) [10] and *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 [11]. Similarly, priming of *Arabidopsis* plants using benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) resulted in altered acetylation and methylation of *AGO2* promoter, which in turn provided enhanced resistance against *Cucumber mosaic virus* [12].

Though regulation of defence genes was known to be modulated by many TFs as mentioned earlier in this chapter, it is interesting to note that epigenetic regulation is involved in modulating these modulators. For example, promoters of three WRKY TFs, WRKY29, WRKY6, and WRKY53 underwent histone methylation and acetylation under biotic stress conditions and some of these modifications facilitated gene expression in primed plants [13].

4.2 Epigenetic modulators under biotic stress

About 130 genes are known to be involved in epigenetic regulation in plants [14]. We selected 60 genes involved in DNA and histone methylation (**Table 1**) and looked whether there are any previous reports on their expressions in the eFP Browser (*Arabidopsis* eFP browser 2.0 – BAR) and this revealed that all the 60 genes invariably had altered expression under many biotic stresses such as infection due to *P. syringae*, *Botrytis cinerea*, *Erysiphe oronti* or *Phytophthora infestans* [30]. Similarly in rice, several genes involved in the RNA-directed DNA methylation (RdDM) pathway showed altered expression under many bacterial pathogens [31]. This indicates that epigenetic regulation in plants is closely associated with biotic stresses.

4.3 Regulators of epigenetic modifications that influence defence genes

Like any other pathway in organisms, epigenetic regulation is also maintained by many key players. There are reports that many such epigenetic regulators are directly associated with the expression of many defence genes as, observed in various loss-of-function mutant plants. JmjC DOMAIN-CONTAINING PROTEIN 27 (JMJ27), an *Arabidopsis* JmjC domain-containing histone demethylase 2 (JHDM2) family protein, was involved in suppressing the expression of three repressors of defence TFs, WRKY25, WRKY26, and WRKY33 in *Arabidopsis* [32]. Promoters of two of these TFs WRKY25 and WRKY33 and, *PR1* gene were found to be hypermethylated in *Arabidopsis* plants deficient for JMJ27 [32, 33]. Two sub-units of the elongator complex protein that interacted with RNA polymerase II, ELP2 [34] and ELP3 [35], also additionally functioned as epigenetic regulators of plant defence genes. ELP2 altered histone acetylation of the promoter of *NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1* (*NPR1*), a key defence gene activator, and regulated the kinetics of the expression of many defence genes [34]. An *Arabidopsis* demethylase encoded by *REPRESSOR OF SILENCING 1* (*ROS1*) was shown to be involved in removing methyl groups from the promoter region of two genes *RECEPTOR-LIKE PROTEIN*

No.	Genes names and category		Function	References
1.	**DNA METHYLATION**	*CMT2*	Plant DNA methyltransferase	[15]
2.		*DRM2*	Maintenance of CHH methylation	
3.		*CMT3*	A chromomethylase involved in methylating cytosine at non-CG sites	
4.		*DDM1*	Gene silencing and maintenance of DNA methylation	
5.		*OTU5*	Phosphate (Pi) homeostasis during DNA and histone methylation	[16]
6.		*MT1*	Maintenance of CG and CHG methylation	[17]
7.		*ROS1*	Acts along with DME	
8.		*DME*	Catalyses the release of 5-methylcytosine (5-meC) from DNA by a glycosylase/lyase mechanism	
9.		*MET1*	Maintains DNA methylation in CHG context	[18]
10.		*NRPD1B*	Major trans acting locus affecting DNA methylation	
11.		*BRU1*	Link between responses to DNA damage and epigenetic gene silencing	[19]
12.		*MRE11*	DNA repair and meiotic recombination	
13.		*FLC*	High level delays flowering unless treated with prolonged cold	[20]
14.		*KYP*	Encodes a histone 3 lysine 9 specific methyltransferase involved in the maintenance of DNA methylation	
15.		*NRPD1*	Required for posttranscriptional gene silencing	[21]
16.		*NRPE1*	Normal RNA directed DNA methylation at non CG methylation sites and transgene silencing	
17.		*MOM1*	Prevents the transmission of stress-induced transcriptional changes to progeny of the stressed plants	
18.		*EDM2*	Prevents ectopic 3′ end processing of mRNA in atypically long introns containing T-DNA sequences	[22]
19.		*RDR2*	Encodes RNA-dependent RNA polymerase that is required for endogenous siRNA (but not miRNA) formation	
20.		*DCL3*	Encodes a ribonuclease III family protein that is required for endogenous RDR2-dependent siRNA formation	
21.		*AGO4*	SiRNA mediated gene silencing, CpNpG and CpHpH methylation	
22.		*NRPD2A*	encodes a shared subunit of RNA polymerase IV and V	
23.		*DRM2*	A putative DNA methyl transferase with rearranged catalytic domains	
24.		*AGO6*	Important for DNA methylation and transcriptional gene silencing	

No.	Genes names and category		Function	References
25.		*DCL2*	Encodes a Dicer-like protein that functions in the antiviral silencing response	
26.		*DCL4*	Encodes an RNaseIII-like enzyme that catalyses processing of trans-acting siRNA precursors	
27.		*LFY*	Encodes transcriptional regulator that promotes the transition to flowering, T DNA shows high methylation	
28.		*LHP1*	Required for epigenetic maintenance of the vernalized state	[23]
29.		*SUVH2*	Encodes a SET domain protein that is involved in epigenetic regulation	
30.		*MEA*	Has a SET domain for methyltransferase activity and is involved in the stable transcriptional silencing of target genes	
31.		*VIM1*	Involved in centromere heterochromatisation, CG methylation	[24]
32.	**HISTONE METHYLATION**	*ATX1*	Activates the expression of AtWRKY70 epigenetically by nucleosomal histone H3K4 trimethylations	[25]
33.		*WRKY70*	ATX1 leads to nucleosomal histone H3K4 trimethylations that activate AtWRKY70, which in turn activates *PR1* and *THI2.1* defence genes	
34.		*WRKY40*	Histone methylations at the AtWRKY40 promoter activate the SA-dependent pathway to control plant immunity	
35.		*FLD*	Epigenetically influences systemic-acquired-resistance induced expression of AtWRKY29 and AtWRKY6 through histone modifications at their promoters	
36.		*SUVH2*	Leads to H3K4me2 and H3K4me3 methylation that epigenetically regulates AtWRKY53 to mediate leaf senescence responses	[26]
37.		*SUVH5*	Histone methyl transferase activity and maintenance of H3 mK9	
38.		*SUVH6*	Binds to methylated cytosine of CG, CNN, CNG	
39.		*ATXR5*	Control of replication of transposable elements and repeated sequences	[27]
40.		*ATXR6*	Control of replication of transposable elements and repeated sequences	
41.		*EMF1*	Maintains vegetative development, encodes a putative transcriptional regulator	[28]
42.		*EMF2*	Maintains vegetative development, encodes a polycomb group	
43.		*PISTILLATA*	Maintains gene silencing via histone modification	
44.		*APETALA3*	Maintains gene silencing via histone modification	
45.		*APETALA2*	Epigenetic maintenance of reproductive development	[29]
46.		*PR1*	Gene involved in plant response to pathogen	[9]

No.	Genes names and category	Function	References
47.	*ASHH2*	Histone methyltransferase involved in di and tri-methylation of 'Lys-36' of histone H3 (H3K36me2 and H3K36me3)	
48.	*ASHR1*	Histone-lysine N-methyl transferase activity	
49.	*TOC1*	Contributes to plant fitness like biomass, carbon fixation by influencing circadian clock period.	[21]
50.	*PRR7*	Essential component of a temperature-sensitive circadian system	
51	*PRR9*	Essential component of a temperature-sensitive circadian system	
52	*HAC1*	Necessary component for bacterial resistance.	
53.	*SUVR4*	One of the four closely related *Arabidopsis* SUVR proteins that belong to the SU(VAR)3–9 subgroup of SET-domain proteins	[24]
54.	*CLF*	Putative role in cell fate determination. Involved in the control of leaf morphogenesis.	
55.	*MEA*	Has a SET domain for methyltransferase activity and is involved in the stable transcriptional silencing of target genes.	
56.	*SWN*	Encodes a polycomb group of protein	
57.	*SDG4*	Contains a SET domain which is known to be involved in modification of histone tails by methylation	
58.	*SDG26*	SET domain, histone methylation	
59.	*ATPRMT4A*	Encodes a type I protein arginine methyltransferase	
60.	*ATPRMT4B*	Encodes a type I protein arginine methyltransferase	

Table 1.
Various epigenetic modulators and their functions.

43 (RLP43) and *RESISTANCE METHYLATED GENE 1* (*RMG1*), which encode for receptors involved in pathogen recognition [36]. *ROS1* antagonised the action of RdDM and interestingly, removed methylation from the binding sites of WRKY TFs of *RLP43* promoter, thereby exposing the site for TF binding [36]. RNA polymerase V, an enzyme required for RdDM, also played a crucial role in differential histone modification of genes involved in SA pathway [37].

Using the *Arabidopsis-P. syringae* pathosystem, it was revealed that three *ASH1-RELATED* (*ABSENT, SMALL OR HOMEOTIC DISCS 1*) genes *ASHR1*, *ASHR3,* and *ASHH2* that were involved in histone methylation, also served the function of compromising resistance in plants [9]. There was decreased histone methylation of *PR1* gene promoter in plants with mutant *ashr1* and *ashh2* alleles [9]. While the avirulent *P. syringae* strain slightly increased the expression of *ASHR1*, *ASHR3,* and *ASHH2* genes in wild plants, an antagonistic response was induced by the virulent strain, indicating interference of pathogen in the expression of genes involved in writing the histone marks. Histone deacetylase 6 (HDA6) was found to bind to the promoters of *PR1* and *PR2* genes, thereby leading to their decreased transcription [38]. Expression of several defence genes and *R* genes was compromised and/or the histone methylation status of their promoters was altered under pathogen stress in

Arabidopsis loss-of-function mutant (*sdg8–1*) for the gene encoding a histone methyltransferase [*SET DOMAIN GROUP8* (*SDG8*)] [39, 40]. H2A.Z, a histone variant was involved in suppressing the expression of multiple SAR genes [8]. Infection of rice plants with the nematode *Meloidogyne graminicola* resulted in differential histone modifications at H3K9 that is associated with plant defence genes [41] MOS9, a protein that regulated two *R* genes, *CONSTITUTIVE 1* (*SNC1*) and *RECOGNITION OF PERONOSPORA PARASITICA 4* (*RPP4*), was found to be associated with a histone methyltransferase Trithorax-Related7 (ATXR7) and, MOS9 and ATXR7 together were involved in the regulation of the two *R* genes using histone methylation [42]. ATPase SPLAYED (SYD) was found to be involved in the chromatin remodelling of promoters of various genes involved in JA and ethylene (ET) signalling pathways [43]. A kind of cyclic interdependence of histone deacetylases (HDACs), SA, and nitric oxide (NO) was discovered in *Arabidopsis*. While NO increased histone acetylation and/or increased expression of many defence genes by adversely affecting HDACs, SA induced endogenous NO, inhibited HDAC activity, and increased histone acetylation [44].

4.4 Epigenetic regulators that provide immunity/resistance/susceptibility

There are several reports confirming that various epigenetic regulators are directly involved in either providing immunity to plants or, to render the plants susceptible to various stresses. For instance, studies on *Arabidopsis* mutants for genes encoding various subunits of RNA polymerases (Pol) IV and V, which are required for RdDM, revealed that Pol V and not IV was involved in host defence [37]. Interestingly, these mutants had enhanced and reduced expressions of genes involved in SA and JA pathways, respectively. As a consequence, the plants were resistant to the bacterial pathogen *P. syringae* and susceptible to the fungal pathogens, *B. cinerea* and *Plectosphaerella cucumerina*. Lack of *JMJ27* rendered *Arabidopsis* mutants more susceptible to virulent *P. syringae* and, the expression of various *PR* genes belonging to *PR1*, *PR3*, *PR4* and *PR5* was found to be compromised in these plants [32]. The *Arabidopsis REPRESSOR OF SILENCING 1* (*ROS1*) was shown to be involved in providing basal resistance towards *Pseudomonas syringae* and facilitated flagellin-triggered immunity [36]. Histone deacetylase 6 (HDA6) was identified as a negative regulator of defence as plants with mutated allele coding for HDA6 exhibited enhanced resistance against the pathogenic strain of *P. syringae* and, constitutively expressed *PR1* and *PR2* genes [38]. Null mutations of genes encoding H2A.Z histone variant in *Arabidopsis* also resulted in enhanced resistance to phytopathogenic *P. syringae* pv. *tomato* [8]. In contrast, loss-of-function *Arabidopsis* mutants of *HISTONE MONOUBIQUITINATION1* (*HUB1*) [45] and *SDG8* [39] exhibited increased susceptibility to two necrotrophic fungi *B. cinerea* and *Alternaria brassicicola*. HUB1 interacted with another protein MED21, which was required for defence against plant necrotrophic fungi [45]. *SDG8* regulated *LAZ5* that encoded an RPS4-like R-protein and as a consequence, the *Arabidopsis* mutants for *SDG8* were compromised for resistance against *P. syringae tomato* DC3000 as well [40]. However, the *syd* mutant *Arabidopsis* were susceptible to *B. cinerea* and not to *P. syringae*, a biotroph [43]. *Arabidopsis* mutants for *KYP* that coded for H3K9me2 methyltransferase exhibited reduced manifestation of the aphid *Myzus persicae* larvae and enhanced expression of aphid-resistance genes [46].

4.5 Biotic stress-induced epigenomic changes

Biotic stress such as microbes and herbivory have induced loci-specific as well global epigenetic changes, both at DNA and histone levels. Infection of *Arabidopsis*

plants with both virulent and avirulent *P. syringae* strain resulted in hypomethylation and major decondensation of centromeric heterochromatin DNA [47]. Virulent and avirulent strains of *P. syringae* and SA treatment-induced differentially-methylated regions across the genome, many of which were associated with altered transcription [48]. This analysis also revealed that SA-induced transposon-associated differently methylated regions were often regulated by siRNA and influenced the transcription of proximal genes. Whole epigenome analysis of *Arabidopsis* plants infected with differentially pathogenic strains of *turnip mosaic potyvirus* (*TuMV*) though indicated no major differences in the induction of methylomes, they exhibited drastic changes in their transcriptomes [49].

Nematode-associated molecular patterns from different nematode species and bacterial pathogen-associated molecular pattern flg22 induced global DNA hypomethylation in rice and tomato plants [50]. Hypomethylation was more common at CHH and not CG or CHG nucleotides in these plants. While herbivory due to an insect *Heliocheilus albipunctella* de Joannis resulted in enhanced global methylation, SA resulted in hypomethylation of *Pennisetum glaucum* genome [51]. Also, the number of larvae significantly reduced in SA-treated plants, indicating an association between altered methylation and defence. In another example of herbivory due to the larvae of aphid *M. persicae* on *A. thaliana*, a global loss of methylation was seen, accompanied by enhanced expression of defence genes [46].

Open chromatin is an indication of epigenetic changes such as histone acetylation, which loosen chromatin. About 10,129 open chromatin sites associated with about 3025 genes, most of which also had enhanced expression, were induced in *Arabidopsis* plants infected with *P. syringae* pv. *maculicola* [52].

A comparison of whole epigenomes in various *Arabidopsis* mutants for genes involved in methylation/demethylation indicated that both these antagonistic processes are required for plant defence [53]. This was understood based on the analysis on four different hypomethylated (*ddm1 F4*, *nrpe1*, *drd1*, and *cmt3*) and two hypermethylated (*ros3* and *ros1*) mutants of *Arabidopsis*, which were infected with the biotrophic fungal pathogen *Hyaloperonospora arabidopsidis*. All the hypomethylated mutants were more resistant compared to the uninfected control, and in contrast, the hypermethylated mutants were more sensitive. While infection with *H. arabidopsidis* lead to enhanced *PR1* expression in *nrpe1* mutant, lack of functional *ros1* lead to suppression of *PR1*. The two opposite mutants *nrpe1* and *ros1* differed in the expression of genes involved in SA and JA pathways. As expected due to SA-JA pathway antagonism, *nrpe1* and *ros1* mutants were more susceptible and resistant, respectively, to the necrotrophic fungi *Plectosphaerella cucumerina*. Transcription of about 49% of pathogen-responsive genes was affected in both *nrpe1* and *ros1*, as described in the same report [53].

The beneficial fungus *Trichoderma harzianum* T22 induced altered global genome methylation status, defence gene expression, and even post-transcriptional regulation in tomato plants [54]. Here, the DNA methylation first decreased and then increased at 24 h and 72 h post-inoculations, respectively.

4.6 Heritable epigenetic biotic stress-induced responses

There are several reports revealing the heritable nature of pathogen-induced epigenetic changes in plants [55, 56]. For example, *Tobacco mosaic virus* (*TMV*) induced global hypermethylation of genomes in *Nicotiana tabacum,* and this change was observed even in the progeny [57]. *Agrobacterium tumefaciens* induced loci-specific changes in *Arabidopsis,* which were retained even in the grand progeny [58]. Pathogen infection also induced heritable resistance in plants. For example, infection with *P. syringae tomato* DC3000 resulted in enhanced resistance against

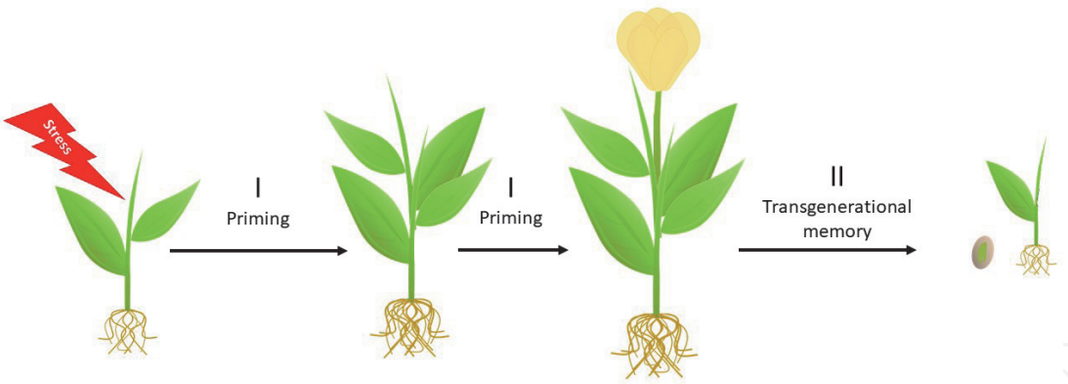

Figure 3.
Transfer of epigenetic memory in plants.

the subsequent infections by PstDC3000 and *H. arabidopsidis* in the progeny *Arabidopsis* plants [59]. Interestingly, altered histone modifications were associated with *PR1*, *WRKY6*, *WRKY53,* and *PLANT DEFENSIN1.2* (*PDF1.2*) of these plants and, the progeny of plants mutant for *NPR1* failed to exhibit this transgenerational defence [59]. Similarly, *TMV* caused hypomethylation of LRR region of the N-gene specific for resistance to *TMV* [57]. Thus, epigenetic memory in plants can be broadly categorised into two, one that retains within the affected generation, and two, that transfers to the progeny (**Figure 3**).

The epiRILs lines of *Arabidopsis* that differ in their DNA methylation patterns but not DNA sequence, have aided in confirming that SA and JA induced heritable phenotypic plasticity, indicating the involvement of heritable epigenetic regulation on plant defence system [60]. Infection of *Arabidopsis* hypo-methylated and hypermethylated mutants *nrpe1* and *ros1*, respectively, with a virulent *Pseudomonas* strain, indicated that NRPE1 and ROS1 could be involved in transgenerational memory [53]. While the progeny of the infected wild-type plants exhibited resistance against another pathogen, *H. arabidopsidis*, *nrpe1* mutants did not show any difference in their resistance level and, *ros1* mutants displayed enhanced susceptibility [53].

Similarly, to pathogens, herbivory also induced transgenerational responses. For example herbivory due to caterpillars in *Arabidopsis* and tomato plants induced reduction in subsequent caterpillar invasion and this priming persisted for two generations [61]. The same group also discovered that *Arabidopsis* mutants deficient in siRNA biogenesis did not inherit this resistance.

The close influence of pathogen stress on epigenetic modification of plant defence system and transgenerational memory offers an entire new array of promises for crop improvement. Approaches of whole epigenome studies under various conditions of biotic stress and resistance would unravel more aspects of the epigenetic regulation of host mechanisms. New avenues of epimutagenic studies that could serve as alternatives for methods that involve gene manipulations/mutations seem to be promising.

5. External application of chemicals or external factors that induce epigenetic changes

There are observations where application of certain chemicals such as SA, JA, methyl jasmonate, systemin, paraquat, abscisic acid, azelaic acid, and pipecolic acid on plants had resulted in enhanced resistance against pathogens, which could even

be heritable [62, 63]. Even conditions of altered salinity, light, drought, and temperature had induced similar results [63]. Reports indicate that the action of some of these external factors involve epigenetic modifications in plants. For example, application of 1-isothiocyanato-4-methylsulfinylbutane on *Arabidopsis* induced chromatin modifications in the two defence genes, *WRKY6* and *PDF1.2*, and reduced the susceptibility to downy mildew disease caused by *H. arabidopsidis* [64]. Thus, such chemicals and other factors provide a non-mutagenic, or the 'epimutagenic' mode of inducing favourable changes in plants that can be used for crop improvement. But since epigenetic stress is highly dynamic and depends on other environmental factors and the stress-pressure [65] careful examination and repeated testing would be required to bring in a commercial-level application of epimutagens.

6. Conclusions

While understanding of the DNA sequence conveyed what information is there in the genome and expression analysis conveyed what information is disseminated, research on epigenetics conveyed how the information is disseminated. While the entire genome of an organism needs to be sequenced only once to get the sequence information, epigenome has to be sequenced and studied multiple times, with multiple approaches, based on the regulatory aspects of relevance. Owing to the dynamic and reversible nature of epigenetic regulation and, phenotypic plasticity, epigenetic regulation can play a crucial role in improvising traits of agronomic importance, including plant defence. Functions of more epigenetic modulators need to be analysed that can tune the plants towards a favourable trait. The function of more epialleles needs to be identified for their application in developing enhanced resistance in plants. With the recent development of non-transgenic method of epigenome editing, epialleles of agronomic importance can be generated and deployed.

Author details

Jasmine M. Shah* and Joyous T. Joseph
Central University of Kerala, Kasaragod, Kerala, India

*Address all correspondence to: jasmine@cukerala.ac.in

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