Innovations in Agriculture 2023, 6:01-07
www.innovationsagriculture.com

ISSN: 2617-1295
DOI: https://doi.org/10.25081/ia.2023-1986

Review Article

Role of epigenetics and the high-throughput sensing techniques to detect stress adaptation mechanisms in crop plants: A mini review

Pronomita Das[1], Dipan Adhikari[2]*

[1]*Indian Institute of Technology, Agricultural and Food Engineering Department, Kharagpur, West Bengal, India*

[2]*UG and PG Department of Botany, Hooghly Mohsin College, Chinsurah, Hooghly- 712101, West Bengal, India*

*Corresponding Author, Email: dipanadhikari@gmail.com

ARTICLE INFO

Received: August 14, 2023
Revised: October 13, 2023
Accepted: October 14, 2023
Published: October 17, 2023

Keywords:

Chromatin; epigenetics, DNA methylation; asymmetric methylations; high throughput sequencing

ABSTRACT

Deciphering the epigenetic code, which will pave the road to understanding the link between genotypic and phenotypic variety, is one of the most fascinating topics of modern genetics. This may significantly contribute to our understanding of the complex mechanisms underlying plants' epigenomic responses to environmental stressors in the context of climate change. As a result of epigenetic regulatory processes such DNA methylation, histone structural modification, and RNA-based regulatory mechanisms, it is hypothesised that chromatin will alter. The interactions of regulatory proteins, including various types of transcription factors, with DNA and chromatin would result in a variety of structural alterations that would impact how genes are expressed. High throughput sequencing techniques have recently been developed to better understand epigenomic alterations in the genome. The use of pharmacological and genetic methods to modify these networks should be made simpler by these strategies. The cytosine methylation of a cell's genomic regions or active cistrons, in particular, is an epigenetic change that these approaches are successful at identifying and studying. To speed up breeding programs for crop improvement, numerous investigations on the interactions between genotype, phenotype, and epigenotype utilising epiGWAS are being carried out. This review will briefly explore the role of epigenetic approaches and high throughput sensing techniques in order to better understand plant stress responses. The value of epigenomic methods and strategies in the nascent "omic" sciences is being emphasized.

INTRODUCTION

Impact of climate change is high on the environment and agricultural crops grown as a result decrease in crop yield. Abiotic stressors are unique to climate change which include drought, rising CO_2, temperature extremes, water logging and other conditions, being known to augment several deleterious influences on a wide range of species. Plants are able to survive these stresses with epigenetic modifications, which are usually followed by changes in gene expression. The aggregate term for a cell's post-translational modifications to histones, biochemical changes to its nucleus DNA, and differences in the production of non-coding RNAs is known as an epigenome. Without changing the underlying base sequence, these alterations usually result in differences in gene expression [1]. Effects of epigenomics on gene expression and genome affect stability through the inhibition or suppression of transcription. DNA methylation influences how genes are expressed under various stress conditions. Expression of genes in response to abiotic stressors is influenced by DNA methylation. Environmental cues have an impact on DNA methylation levels, either negatively in hypomethylation or positively in the case of hypermethylation. The varying methylation patterns of numerous asymmetric methylation sites, such as CNN, CNG, and CG (cytosine methylation sites) by DRM2 and CMT3, also have an effect on stress. Histone modifications are necessary for both stress reactions and plant development. Histone tail phosphorylation, ubiquitination, and acetylation are the mechanisms that mediate gene up-regulation; de-acetylation and biotinylation are the techniques that mediate gene down-regulation. In various ways, plants dynamically alter their histone tails in response to external stresses. The significance of these transcripts in the defense against stress is shown by the accumulation of numerous additional antisense transcripts, a source of siRNAs, in response to abiotic stresses.

Stress causes plants to develop either transgenerational inheritance of Epialleles, which are either transient or permanent epigenetic stress memories, and the origins of epigenetic diversity. Plants evolve and adapt as a result of the stable memory they maintain after a stress has passed, either for the duration of their remaining developmental cycles or by passing it on to the next generation. Most of the transient epigenetic alterations induced by stress revert to their original forms after the stress subsides. Some of the changes, though, may hold true during several meiotic or even mitotic cell cycles. Epialleles are commonly caused by non-genetic or generic sources. Epialleles may arise spontaneously as a result of erroneous methylation state maintenance, short RNA off-target effects, or other non-genetic causes. Epigenetic variation may also have non-genetic sources, such as developmental or environmental factors that affect chromatin directly or indirectly. Two genetic sources of epialleles are transposition insertions, which alter local chromatin, and structural rearrangements, such as alterations in copy number that are either genetically related or unrelated. To use epigenetics to crop breeding, it is critical to generate epigenetic variation as well as identify and evaluate epialleles. It might be required to use epigenome editing or epi-genomic selection in order to generate and identify epialleles. These epigenetic systems are crucial in overcoming the difficulties posed by shifting surroundings [1]. Plants provide a habitat where a variety of interactions between various stressors can take place. The most promising functional genomics approach now is epigenetics, which has enormous potential for enhancing plant response to biotic and abiotic stressors. Plant epigenetics has generated a lot of attention in this context as advances in plant molecular biology have significantly altered our knowledge of the molecular mechanisms that might influence these relationships [2-4].

The epigenome, which is made up of chemically altered DNA and histone proteins, affects tissue differentiation, transposable element suppression and gene expressions during different phases of plant growth and development. Waddington coined the term "epigenetics" in the 1940s [5], defining it as "Gene interactions that effect the

phenotypic alterations." The word "epigenetics" later evolved to refer to the study of heritable changes in gene expression brought on by mitosis and meiosis but without altering the DNA sequence [6]. DNA methylation, chromatin alterations brought on by modifications in histone architecture, and RNA-based regulatory mechanisms are three different types of epigenetic regulation of gene expression. One of the most important areas of study in plant functional genetics has emerged as epigenomics. Because plants are sessile, environmental stressors have an impact on their growth and development. In order to deal with stressful situations, plants have evolved a variety of stress-signaling mechanisms. Among which epigenetic stress sensing is essential for coping with external stress. The finer details and active ingredients underlying plant epigenetics that regulate stress adaptations in various plant communities have recently been the subject of investigation by a number of researchers.

EPIGENETICS AND EPIGENOMICS

Plants can respond to environmental changes thus bringing forth several epigenetic modifications of chromatin structures such DNA methylation, chromatin modifications, and RNA-based regulatory mechanisms. [7].

DNA methylation

By adding a methyl (-CH3) group to the cytosine's fifth carbon, DNA methylation results in the production of 5-methylcytosine. Adenine methylation of DNA, for example, often occurs in prokaryotes, whereas cytosine methylation primarily occurs in both plants and animals (primarily eukaryotes) [8]. By enabling bacteria to distinguish between host's genomic DNA (self) and phage DNA (non-self), bacterial DNA methylation facilitates the breakdown of phage DNA by restriction enzymes produced by the host.DNA methylations are remarkably conserved biological processes in fungi, mammals, and plants. DNA methyl-transferases in plants catalyse three distinct DNA methylation sequences, CpNpG, CpG, and CpNpN (where N = A, C, or T). It may be kept after replication because of the symmetry between CpNpG and CpG methylation [6].

Enzymes involved in DNA methylation

Bases undergo these reversible, enzyme-mediated epigenetic changes. DNA demethylation enzymes and DNA methyl-transferase are the two classes of enzymes that control DNA methylation. DNA Methyl-Transferase 1 (MET1), Chromomethylase 3, and the other class, which comprises the DNA methyl-transferases DRM1 (Domain Rearranged Methylase 1) and DRM2 are the three distinct classes of enzymes that control cytosine methylation in plants [9]. Cytosine methylation (CpG) of the genome is maintained by DNA methyl-transferase 1 (MET1). MET1 is a mammalian methyl-transferase homologue. Contrarily, chromomethylase3 aids in bringing a methyl group to a CHG motif in both transposons and centromeric repeats. The third class, including two DNA methyltransferase, catalyses asymmetrical cytosine methylation at CpNpNp site and de novo methylation [10].

Regulation of DNA methylation

Various developmental, physiological and stress influences DNA methylation in plants. Histone and DNA methylations are correlative mechanisms.This methylation mechanism establishes a condensed state of chromatin called heterochromatinin the downstream of CpG(most likely H3K9methylation) [8]. Demethylation takes place in both active and passive ways. Active demethylation occurs by glycosylase activity which removes the methyl-cytosines in DNA and passive demethylation is the outcome of the parental imprint's inability to be maintained following DNA replication due to de novo methylation inhibition. The formation of persistent hypermethylated epialleles in the plant genome is dramatically inhibited by active demethylation [6] (Fig. 1).

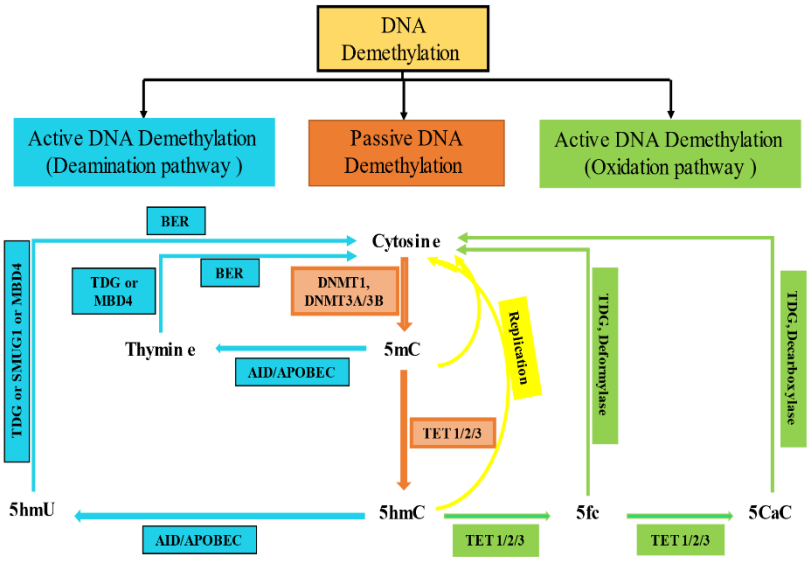

Fig. 1. Graphical representation of active and passive demethylation. Passive DNA demethylation is caused by a reduction in activity or absence of DNMTs (orange arrows). Active demethylation via oxidation pathway (greenarrows): TET enzymes can hydroxylate methylcytidine (5mC) to form 5-hydroxymethylcytosine (5hmC); further oxidation produces 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). 5fC and 5caC can be actively removed by the DNA glycosylases. In addition, a putative Deformylase may convert 5fC to C and decarboxylase convert 5caC to C. Active demethylation via deamination pathway (blue arrows): AID/APOBEC family members can deaminate 5mC or 5hmC to form thymidine or 5-hydroxymethyluracil (5hmU). These intermediates are replaced by cytosine during base excision repair (BER) mediated by the uracil-DNA glycosylase (UDG) family, like TDG or SMUG1 as well as MBD4 (specifically recognize thymine and 5hmU). AID activation-induced deaminase. APOBEC apolipoprotein B mRNA-editing enzyme complex, BER— base excision repair, DNMT1/3A/3B—DNA methyltransferase, MBD4—methyl-binding domain protein 4, SMUG1—single-strand specific monofunctional uracil-DNA glycosylase, TET1/2/3—ten- eleven methyl cytosine dioxygenase family, TDG—thymine-DNA glycosylase.

Chromatin modifications

Covalent post-translational alterations of the histone protein core's N-terminal tail including amino acid residues such as arginine, lysine, threonine, and serine are known as chromatin modifications. These covalent modifications are of mainly two types like acetylation and methylation which are regulated by histone modifying enzymes. Acetylation is regulated by acetyl-transferase and de-acetylase, on the other hand, methylation is regulated by methyl transferase and demethylase. By changing the chromatin state from loose euchromatin to condensed heterochromatin structure or vice versa (Fig. 2), histone changes control the transcriptional activity of a gene [11].

Regulation of chromatin modifications

Histone acetyl-transferase (HATs) positively regulates transcription by acetylating 4, 9, 27, 36, and 73 H3 and H4 lysine (K) positions. In contrast, on the contrary, histone deacetylase (HDACs) negatively control transcription by removing acetyl groups from histone backbones. Histone methylation affects transcription depending on the degree and position of methylation. Lysine and arginine methyl-transferases are two methyl-transferase types that direct lysine (K) and arginine (R) methylation, respectively. H3K48me, H3K79me, H3K36me, and H3K4me3 activate the transcription, and in contrast, transcription repression is directed by H3K9, H3K27, H4K20, and H4R3me2. Another type of histone modification constitutively activates the transcription, like H3 phosphorylation at serine and threonine histone residues [12].

RNA- based control mechanisms

Wasseneger was the first to identify RNA-based regulatory mechanisms in plants, which he called RNA-directed DNA methylation (RdDM). De novo DNA methylation at CHG, CG, and CHH sites is guided by RNA-based regulatory mechanisms in a sequence-specific manner [13].

Mechanism and regulation of RNA -based control mechanisms

The production of double-stranded RNAs (dsRNAs) is the initial step in the synthesis of siRNA. DsRNAs are probably produced by transposable elements, viral replication intermediates, and transcribed inverted repeats. The initial stage in the process of RNA-directed DNA methylation is the production of single-stranded RNA (ssRNA), which is carried out by RNA polymerase IV (RNA POL IV). The next step is catalysed by chromatin remodeler CLASSY 1 (CLSY1, remodelling factor) and RNA-dependent RNA polymerase 2 (RDR2). Using ssRNA as a template, RDR2 produces dsRNA. Small interfering RNA (siRNA) is produced when DICER LIKE 3 (DCL3 proteins) cleaves dsRNA to make it. This siRNA also contains 3' overhangs and is 24 nucleotides long. A single-stranded methylation siRNA is used by ARGONAUTE 4 (AGO 4) to build the AGO4 complex, an RNA-induced silencing complex (RISC). The RISC-AGO4 complex then continues to control methylation at homologous loci. Additionally, at the target site, the DDR complex (DRD1 [DEFECTIVE IN RNA DIRECTED DNA METHYLATION 1], DMS3 [DEFECTIVE IN MERISTEM SILENCING 3], RDM1 [REQUIRED FOR DNA METHYLATION 1], and DMS4]) is required for PolV to transcribe long non-coding RNA (lncRNA). DDR reportedly unwinds DNA in preparation for transcription. The base-pairing between lncRNA and siRNA creates the interaction between the (RISC)-AGO4 complex and PolV. Through interactions with PolV-Nuclear RNA Polymerase E1 (NRPE1), NRPE2 and KTF1 subunits (Kow domain-containing transcription factor), this link is maintained. Cytosine methylation can occur at the target site because to RDM1's interaction with AGO4 and DRM2 (de novo methyltransferase) [13] (Fig. 3).

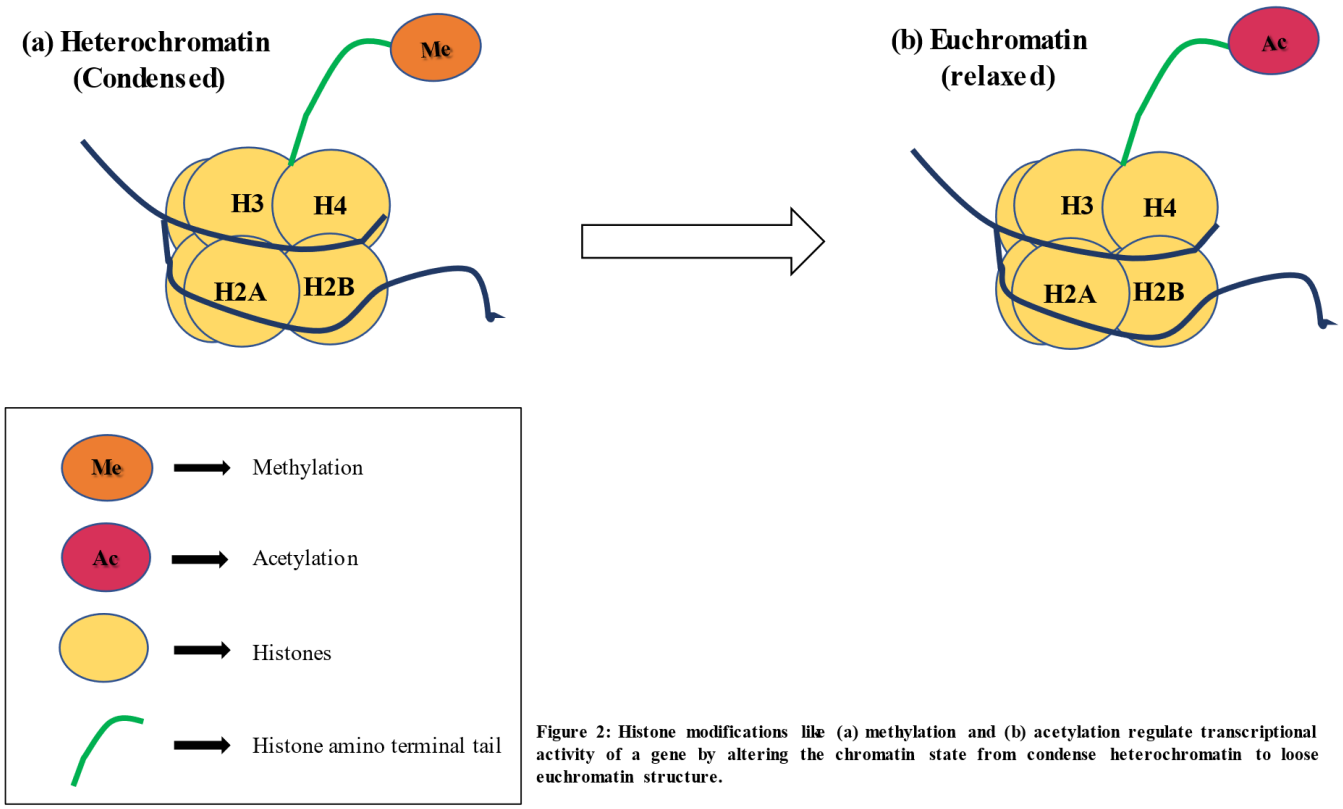

Figure 2: Histone modifications like (a) methylation and (b) acetylation regulate transcriptional activity of a gene by altering the chromatin state from condense heterochromatin to loose euchromatin structure.

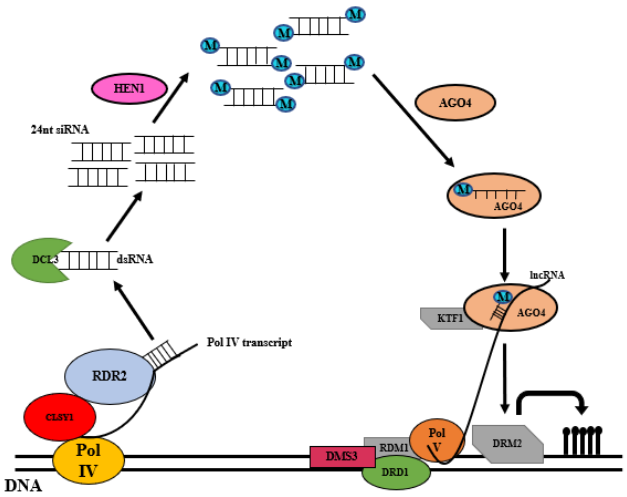

Fig. 3. Pictorial representation of the mechanism of RNA – directed DNA methylation (RdDM) – Firstly, RDR2 interacts with POL IV and converts POL IV transcripts to double stranded RNA (dsRNA) with the help of chromatin remodeler CLSY 1. The dsRNAs further forms 24-nt siRNAs by DCL3 action, and the guide strand is loaded onto AGO4, which then enters the POL V-mediated pathway of de novo DNA methylation.

Epigenetic stress memory and its detection tools

The transgenerational inheritance of DNA methylation patterns in plants has been proposed by researchers, and it is suggested that this inheritance may aid in the tolerance of environmental stress. Epigenetic controls aid in both long-term stress adaption and an organism's immediate reaction to stress reduction. Different rice cultivars (Oryza sativa) grown under alkaline and salt stress were reported to preserve altered DNA methylation in selfed offspring. Short-term stress adaption has also been linked to these phenomena. For instance, in stress-treated plant progenies, global hypermethylation could be observed in the absence of stress, although these epigenetic effects had not persisted in subsequent generations. Using a stress-treatment experiment, the amount of cytosine DNA methylation in two generations of Arabidopsis has been measured [14]. Both treated and untreated Arabidopsis progeny plants had higher levels of 5mC. When there is no strep, DNA methylation falls in unaffected plants of the same generation.

Genome wide epigenetic analysis by sequencing technology

Chromatin immunoprecipitation-mediated high throughput sequencing (ChIP-sequencing), bisulphite conversion, methylation-sensitive restriction enzyme digestions, and small RNA-mediated methylation are four categories of genome-wide analytical techniques that can be used to identify patterns of DNA methylation and chromatin modifications in the genome. The basis for categorising these groupings is the difference between DNA methylation and demethylation. Promoters are often CpG-rich areas in the genome that are methylated [15]. A hypermethylated state in the promoter regions with CpG islands is shown to be influenced by a variety of environmental circumstances, inactivating the problematic genes. Consequently, it is useful to quantify the differentially methylated regions (DMRs) in the plant genome during various stress responses.

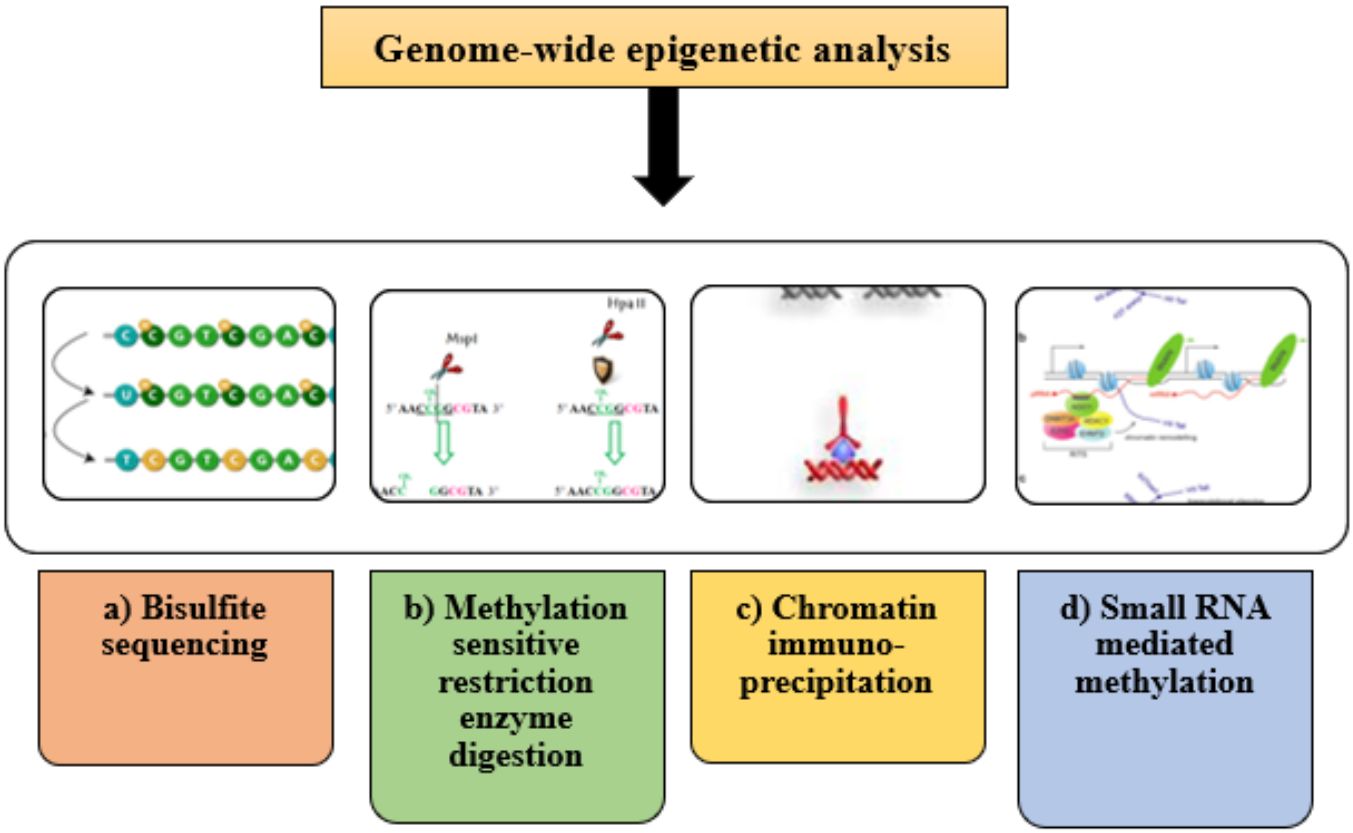

Fig. 4. Genome-wide epigenetic analysis by sequencing technology in use.

Bisulfite sequencing

The sodium bisulfite treatment used in the bisulfite sequencing method causes unmethylated cytosine in genomic DNA to change into uracil, but it has no effect on methylated cytosine residues. Then, using sequence-specific primers, PCR amplification of the transformed DNA is carried out, which aids in determining the DNA methylation status. In order to discover specific alterations in the genomic DNA sequence, bisulfite treatment can thus offer interesting information. A DNA sequence's methylation status can be determined at the single nucleotide level using this information, which is dependent on the methylation circumstances. This ensures the recovery of the data for nucleotide resolution using a number of bioinformatic techniques. Understanding the degree of methylation in the active cistron's UTRs (untranslated regions), promoters, and other protein-coding regions can also be aided by whole genome bisulfite sequencing. It can also be used to quantify short RNAs, sequence transcriptomes, and analyse their relationships to the frequency of DNA methylation [16]. Strand-specific mRNA sequencing can reveal changes in the number of transcripts in various genomic regions, including intergenic regions, transposons, and gene changes in the numerous transcripts of transposons, hundreds of genes, and unannotated intergenic transcripts. In a nutshell, each of these data sets contributes to our understanding of the intricate epigenetic connection between DNA methylation and transcription. At present a large amount of research focus has been concentrated onwhole genome bisulfite sequencing in generating methylomes maps ranges from Arabidopsis to Zea mays. A different, efficient, and less expensive method of bisulfite sequencing called reduced-representation bisulfite sequencing (RRBS) was developed [17] and is used to study how the mammalian methylome is regulated. The RRBS method digests the genome using the Msp1 restriction enzyme, which is subsequently transformed into bisulfite [30]. Next-generation sequencing is then used to ascertain the methylation patterns of specific segments [31]. There are a number of bioinformatic analyses that can be used in conjunction with web-based tools to support the quantitative analysis of bisulfite sequencing data derived from plant methylation status. For instance, researchers [18] created methylKit, a user-friendly tool with a multi-threaded R package that has the ability to quickly analyse and characterise data from a group of methylation studies. Similar to this, researchers [19] created Methy-Pipe, another pipeline for methylome analysis that is effective for both upstream and downstream processing of methylation data. A versatile tool (Bismark) for the examination of bisulfite sequencing data was found [20].

Methylation-sensitive enzyme restriction digestion

Methylation-sensitive amplified polymorphism (MSAP) is a technique for determining DNA methylation without knowing the DNA sequence. This method aids in determining the pattern of cytosine methylation in genomes.

This identification approach uses two isoschizomers that are methylation-sensitive, such as Hpa II and Msp I. Their function differs in their methylation level sensitivity to some recognition sequences (5`-CCGG-3`). Methylated strands (both) of double stranded cytosine methylations disables HpaII restriction but enables the restriction activity when external cytosine is hemimethylated [21]. On the contrary, MspI actively cuts fully or hemi-methylated C5mCGG but not 5mCCGG. Based on restriction sites, it is possible to analyse the locus-specific distinction between DNA sequences that have been methylated and those that have not. Recently, the MSAP approach has been used to investigate the methylation/demethylation state of plants. By comparing the methylation pattern in various soybean cultivars, many research groups use this quantitative analysis of differentially methylated areas in agronomic variables in soybeans [22].

Chromatin immunoprecipitation

Epigenomic dynamic alterations of chromatin are diverse for developmental stages, disease states and distinct tissue types for different environmental stress responses. Genome wide high throughput technologies are used to analyse these chromatin states or epigenetic phenomena [23]. Chromatin immunoprecipitation, often known as ChIP assays or ChIP sequencing, has recently become the method of choice for studying epigenomics [24]. ChIP seq discovers genome wide modifying state of chromatin complex along with transcription factors and other proteins. The approach analyses the regulation of histone or other DNA-protein interactions in different cell types, developmental stages and environment effects. The ChIP seq method includes cross-linking, isolation and chromatin fragmentation along with protein-DNA complex capturing by antibodies against the transcription factors or histone proteins. The immunoprecipitated protein-DNA complexesare reversely cross-linked. The purified DNA can be used for additional investigation by being hybridised to microarrays, such as high-throughput sequencing (ChIP-seq) or ChIP-chip assay [24, 25].

This method entails extracting and fragmenting chromatin after formaldehyde cross-links with the histones and DNA-protein nucleosome complexes. Finally, such dispersed samples are approved for chromatin immunoprecipitation (ChIP) using modification-specific antibodies. Then PCR amplification can be done to obtain proper amount of DNA which then undergoes denaturation to produce the single stranded DNA (ssDNA). These ssDNAs are fluorescent-labelled for sample differentiation. Lastly, on the DNA microarray surface that represents the genomic region of interest, fluorescently tagged fragments are hybridised with the target single stranded sequences [26, 27]. The chip array's complementary tagged fragments and the target sequences would then combine to generate dsDNA, which will then be illuminated by fluorescence. Finally, the fluorescent signals are generalized with administrate signals along with statistical tools to check out the methylated regions. Then to find the physical positions, reference genome can be used for mapping the coordinates of microarray probes.

The use of next-generation sequencing of ChIP fragments to produce high-resolution, high genome coverage, and low noise results in comparison to ChIP-on-chip tests is a potent method. The size and compactness of the chromatin fragments utilised for ChIP as well as the probes on the array are also factors that affect the resolution of ChIP-on-chip. Contrarily, the generation of the same fragment size and the specifics of the reads during sequencing determine the resolution of ChIP-seq [28]. ChIP-seq is relatively cost-effective in achieving plant genome derived nucleosome complexes resolution than ChIP-on-chip assay.

Small RNA mediated methylation

Small interfering RNA (SiRNA) can direct chromatin to genomic areas with a similar sequence. The size of the total RNA population from various genotypes, tissue types, mutations, and subspecies will determine the epigenomic analysis approach that is most acceptable. After total RNA isolation from the appropriate samples, size fractionation is carried out. Then, at both of the sRNA ends, which serve as primer binding sites during PCR amplification and reverse transcription, DNA adaptors would be ligated. NGS sequencing can then be performed, followed by large-scale reads covering the entire genome [6, 29].

CONCLUSION

A deeper comprehension of the mechanisms governing epigenetic expression in crop plants will aid in unravelling the relationships between environment and genotype, qualitatively recognising the effects of global climate change on adaptability and, ultimately, on the crop productivity. The creation of crop methylomes would also make it possible to create novel molecular markers associated with epigenetic modifications that will aid in plant breeding. Recent genetic profiling techniques that enable the diagnosis of temporary stress situations in order to control plant stresses in a sustainable and careful manner may be related with next-generation markers. To give a direction of thought about the correlation between genetic and phenotypic variance, it is actually much more necessary to propose the existence of a "epigenetic code" in addition to the well-known genetic code [32]. The primary forces behind the epigenetic coding are DNA methylation in the CG, CH, and CHH regions. Histone methylations, such as H3K27me2/3 that happen in response to bacterial stress, should be the driving force behind other epigenetic mechanisms controlled by chromatin rearrangement. Keep in mind that epigenetic differences frequently link to the underlying genetic variants; therefore, both of these factors should be researched simultaneously.

FUTURE PERSPECTIVES

The investigation of epigenetic inheritance in plants is currently in high demand. Epigenetic research may result in the development of novel and improved methods for crop development and environmental stress tolerance. Technologies that are rapid and effective are now available to study both genotype and epigenotype. These assist in putting together critical hints and leads for research on how epigenetics affects important phenotypes and adverse responses to environmental cues. Gene activity is a crucial biochemical marker of environmental stress responses linked to development, cell fate determination, and cellular proliferation. Gene activity is regulated by epigenetic mechanisms such DNA methylation, short RNAs, and chromatin changes. This way of thinking makes it possible to identify the DNA methylation and sRNA processes that lead to aberrant phenotypes by using gene allele mutations. On the other hand, this finding allows the researchers to now concentrate on the appropriate genes, genomic regions, and transcriptional factors to produce the required phenotypes for further crop enhancement programs. The epigenetic mechanisms underpinning chromatin changes and the resulting transcriptional regulation that affect how plants respond to environmental stresses must be further studied in order to gain a better understanding of these processes. The genetic stress memory system also needs more research.

ACKNOWLEDGEMENT

The authors are deeply indebted to express their gratitude to the PG dept of Botany, Hooghly, Mohsin College and Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur, West Bengal, India, for their overwhelming support for providing all necessary support and library facilities and assistance to write this paper.

AUTHOR CONTRIBUTIONS

Both Dr. Dipan Adhikari and Miss. Pronomita Das have contributed equally to shape up this paper. Dr. Adhikari conceptualized and formulated the blueprint while Miss. Pornomita Das has written the paper. Dr. Adhikari made the editing and necessary corrections for the final format.

REFERENCES

1. Kumar M, and Rani K. Epigenomics in stress tolerance of plants under the climate change. Mol Biol Rep. 2023;50(7):6201–6216. https://doi.org/10.1007/s11033-023-08539-6.
2. Saeed F, Chaudhry UK, Bakhsh A, Raza A, Saeed Y, Bohra A and Varshney RK, Moving beyond DNA sequence to improve plant stress responses. Front Genet. 2022; 13:874648. https://doi.org/10.3389/fgene.2022.874648.
3. Miryeganeh, M. Plants' Epigenetic mechanisms and abiotic stress. Genes. 2021; 12, 1106. https://doi.org/10.3390/genes12081106.
4. Singroha G and Sharma P. Epigenetic modifications in plants under abiotic stress, epigenetic modifications in plants under abiotic stress, IntechOpen. 2019. DOI: http://dx.doi.org/10.5772/intechopen.84455.
5. Tronick E, Hunter RG, Waddington, dynamic systems, and epigenetics. Front Behav Neurosci. 2016;10(10):107. doi: 10.3389/fnbeh.2016.00107.
6. Yadav CB, Pandey G, Muthamilarasan M, Prasad M. Epigenetics and Epigenomics of Plants. Adv Biochem Eng. 2018;164:237–261. https://doi.org/10.1007/10_2017_51.
7. Handy DE, Castro R, Loscalzo J. Epigenetic modifications: basic mechanisms and role in cardiovascular disease. Circulation. 2011;17;123(19):2145-56. doi: 10.1161/CIRCULATIONAHA.110.956839.
8. Moore LD, Le T, and Fan G. DNA methylation and its basic function. Neuropsychopharmacol. 2013;38(1):23-38. doi: 10.1038/npp.2012.112.
9. Zhong X, Du J, Hale CJ, Gallego-Bartolome J, Feng S, Vashisht AA, Chory J, Wohlschlegel JA, Patel DJ, Jacobsen SE. Molecular mechanism of action of plant DRM de novo DNA methyltransferases. Cell. 2014; 157(5):1050-60. doi: 10.1016/j.cell.2014.03.056.
10. He XJ, Chen T, Zhu JK. Regulation and function of DNA methylation in plants and animals. Cell Res. 2011;21(3):442-65. doi: 10.1038/cr.2011.23.
11. Smith BC, and Denu JM. Chemical mechanisms of histone lysine and arginine modifications. Bioch Biophys Acta. 2009;1789(1),45–57. https://doi.org/10.1016/j.bbagrm.2008.06.005.
12. Kumar V, Thakur JK, Prasad M. Histone acetylation dynamics regulating plant development and stress responses. Cell. Mol. Life Sci. 2021;78;4467–4486. DOI: https://doi.org/10.1007/s00018-021-03794-x.
13. Erdmann RM, Picard CL. RNA-directed DNA Methylation. PLoS Genet. 2020;8:16(10):e1009034. doi: 10.1371/journal.pgen.1009034.
14. Boyko A, Blevins T, Yao Y, Golubov A, Bilichak A, Ilnytskyy Y, Hollunder J, Meins F Jr, Kovalchuk I. Transgenerational adaptation of Arabidopsis to stress requires DNA methylation and the function of Dicer-like proteins. PLoS One. 2010;5(3):e9514. doi: 10.1371/journal.pone.0009514.
15. Moore L, Le T. and Fan G. DNA Methylation and Its Basic Function. Neuropsychopharmacol. 2013; 38, 23–38 https://doi.org/10.1038/npp.2012.112.
16. Parent JS, Cahn J, Herridge RP, Grimanelli D, Martienssen RA. Small RNAs guide histone methylation in *Arabidopsis* embryos. Genes Dev. 2021;35(11-12):841-846. doi: 10.1101/gad.343871.120.
17. Paun O, Verhoeven KJF, Richards CL. Opportunities and limitations of reduced representation bisulfite sequencing in

plant ecological epigenomics. New Phytol. 2019;221(2):738-742. doi: 10.1111/nph.15388.
18. Akalin A, Kormaksson M, Li, S. et al. methylKit: a comprehensive R package for the analysis of genome-wide DNA methylation profiles. Genome Biol. 2012;13,R87. https://doi.org/10.1186/gb-2012-13-10-r87.
19. Jiang P, Sun K, Lun FMF, Guo AM, Wang H, Chan KCA, et al. Methy-Pipe: An Integrated bioinformatics pipeline for whole genome bisulfite sequencing data analysis. PLoS ONE. 2014;9(6): e100360. https://doi.org/10.1371/journal.pone.0100360.
20. Krueger F, Andrews SR. Bismark: a flexible aligner and methylation caller for Bisulfite-Seq applications. Bioinformatics. 2011;27(11),1571–1572. https://doi.org/10.1093/bioinformatics/btr167.
21. Fulneček J and Kovařík A. How to interpret methylation sensitive amplified polymorphism (MSAP) profiles? BMC Genet. 2014;15: 2. doi: 10.1186/1471-2156-15-2.
22. Shen Y, Zhang J, Liu, Y. et al. DNA methylation footprints during soybean domestication and improvement. Genome Biol. 2018;19:128 https://doi.org/10.1186/s13059-018-1516-z.
23. Kidder BL, Hu G, Zhao K. ChIP-Seq: technical considerations for obtaining high-quality data. Nat Immunol. 2011;12(10):918-22. doi: 10.1038/ni.2117.
24. Canton M, Farinati S, Forestan C. et al. An efficient chromatin immunoprecipitation (ChIP) protocol for studying histone modifications in peach reproductive tissues. Plant Methods. 2022;18,43 https://doi.org/10.1186/s13007-022-00876-0.
25. Mundade R, Ozer HG, Wei H, Prabhu L, Lu T. Role of ChIP-seq in the discovery of transcription factor binding sites, differential gene regulation mechanism, epigenetic marks and beyond. Cell Cycle. 2014;13(18):2847-52. doi: 10.4161/15384101.2014.949201.
26. Trevino V, Falciani F. and Barrera-Saldaña HA. DNA Microarrays: A Powerful Genomic Tool for Biomedical and Clinical Research. Mol Med. 2007;13,527–541 https://doi.org/10.2119/2006-00107.Trevino.
27. Milne TA, Zhao K, Hess JL. Chromatin immunoprecipitation (ChIP) for analysis of histone modifications and chromatin-associated proteins. Methods Mol Biol. 2009;538:409-23. doi: 10.1007/978-1-59745-418-6_21.
28. Mahony S. and Pugh BF. Protein-DNA binding in high-resolution. Crit Rev Biochem Mol Biol. 2015;50(4):269-83. doi: 10.3109/10409238.2015.1051505.
29. Li C, Lin F, A D, Wang W, Huang R. Genome sequencing and assembly by long reads in plants. Genes. 2017;28:9(1):6. doi: 10.3390/genes9010006.
30. Yuanxin Xi, Christoph Bock, Fabian Müller, Deqiang Sun, Alexander Meissner, Wei Li., RRBSMAP: a fast, accurate and user-friendly alignment tool for reduced representation bisulfite sequencing, Bioinformatics. 2012;28(3):430–432. https://doi.org/10.1093/bioinformatics/btr668.
31. Lou S, Lee HM, Qin H, Li JW, Gao Z, Liu X, Chan LL, Lam, KV, So WY, Wang Y, Lok S, Wang J, Ma RC, Tsui SK, Chan JC, Chan TF, and Yip KY. Whole-genome bisulfite sequencing of multiple individuals reveals complementary roles of promoter and gene body methylation in transcriptional regulation. Genome biology. 2014;15(7):408. https://doi.org/10.1186/s13059-014-0408-0.
32. Perrone A, Martinelli F. Plant stress biology in epigenomic era. Plant Sci. 2020;294:110376. doi:10.1016/j.plantsci.2019.110376.