SINET: *Ethiop. J. Sci.*, 47(3): 258-286, 2024 ISSN: 0379–2897 (PRINT)
© College of Natural and Computational Sciences, Addis Ababa University, 2024 eISSN: 2520–7997

Date received: November 11, 2024; Date revised: September 14, 2024; Date accepted: September 31, 2024
DOI: https://dx.doi.org/10.4314/sinet.v47i3.8

Review on Epigenetic Modification Mechanisms and their Application for the Genetic Improvement of Plants

Leyila Nasir *, Teslim Yimam and Demsachew Guadie

Institutes of biotechnology, Addis Ababa University, Ethiopia. E-mail: leylanasir2012@gmail.com

ABSTRACT: The study of epigenetics is concerned with the changes in gene expression that occur without altering DNA sequences. DNA methylation, histone modification, and RNA interference are the major epigenetic mechanisms that play a transgenerational role in organism development. These modifications are dynamic and reversible, which makes them significant to regulating gene expression, growth, and development in plants. DNA methylation emerges as one of the most important molecular mechanism and it has a complex process through the involvement of DNA methyl transferases and ten-eleven translocations (TET). It has a great role in plant stress responses and crop improvement. Histone modification is the second regulatory mechanisms that modulate the chromatin structure and thereby affect various processes, such as gene transcription, DNA replication, DNA recombination, and DNA repair in cells. It is involved in different plant responses to biotic and abiotic stresses. The exploration of non-coding RNAs brings attention to their roles in post-transcriptional gene regulation. Small RNA-mediated epigenetic modification is a potential in understanding and manipulating plant gene expression. By understanding these epigenetic mechanisms, researchers can develop innovative strategies to improve crop yield, quality, and stress tolerance. This review aims to provide a comprehensive overview of epigenetic mechanisms in plants and their potential applications in agriculture. By exploring the intricate interplay between genetic and epigenetic factors, we can unlock the full potential of plants to meet the growing global food demand and adapt to changing environmental conditions.

Keywords/ phrases: Crop improvement, DNA methylation, Epigenetics, Histone modification RNA interference, Stress resistance

INTRODUCTION

Epigenetics is a field in molecular biology that explores the complex mechanisms, which control variations in gene expression without changing the fundamental sequence of DNA nucleotides (Kumari *et al.*, 2022). The science of molecular alterations in and around DNA has revealed a fascinating tapestry that affects the reading and interpretation of genetic information within cells. In contrast to conventional genetics, which focuses on the nucleotide order in DNA, epigenetics illuminates the dynamic and reversible changes that play a part in the intricate regulation of gene expression (Kumari *et al.*, 2022).

The DNA of eukaryotes is extremely condensed and closely connected with proteins called histones and this combination is referred to as chromatin. A gene's chromatin needs to be accessible for transcription factors (TF) and RNA polymerase binding in order for transcription to begin at that place. Therefore, the chromatin state at a particular gene determines whether the gene is "on" or "off" (Gupta and Salgotra, 2022). Numerous processes, including methylation of DNA, post-translational changes of histones, chromatin remodeling, and non-coding RNAs affect how accessible chromatin is to the transcriptional machinery. Genes that have been generated can have their chromatin state and expression pattern preserved over many generations; this phenomenon is known as epigenetics (Kakoulidou *et al.*, 2021).

The fundamental principle of epigenetics is that these alterations are not only important within the context of a single organism, but also have the potential to be inherited, thereby transmitting regulatory information onto subsequent generations. The significant effect that epigenetic mechanisms can have on an organism's growth, health, and function is highlighted by this transgenerational feature (Chen *et al.*, 2022).

Ensuring future food availability requires a global strategy aimed at minimizing significant crop losses through the optimization of crop protection and resilience against biotic and abiotic stresses. The selection of superior genotypes through epigenetics research and the emergence of improved adaptation will be crucial for crop sustainability in the future in order to meet these biotic and abiotic restrictions (Mercé *et al*., 2020).

Epigenetic mechanisms can be employed to promote the growth and development of plants and make them more tolerant to environmental stressors by controlling gene expression and influencing a variety of plant properties (Kajal *et al*., 2021). Moreover, epigenetic alterations are useful tools for genetic manipulation and plant breeding since they can alter gene expression heritably without altering the primary DNA sequence (Spillane and McKeown, 2020).

Apart from their possible application in genetic enhancement and plant breeding, epigenetic alterations can also be highly helpful in assisting plants in adapting to both biotic and abiotic stresses (Crowl *et al*., 2024). Epigenetic variety has the potential to improve plants' capacity for adaptation and survival in dynamic environments by controlling gene expression, altering plant development, and influencing physiological responses to external stimuli (Kakoulidou *et al*., 2021).

Therefore, understanding the mechanisms underlying plant epigenetic regulation is essential to design crop improvement program to boost crop yields and strengthen plant resistance to environmental stresses like climate change (Qi *et al*., 2023). This review aims to comprehensively examine the intricate mechanisms of epigenetic modifications in plants, specifically DNA methylation, histone modification, and RNA interference. By elucidating how these epigenetic processes regulate gene expression, growth, and development in plants, with a particular emphasis on their roles in stress responses and crop improvement strategies, we aim to highlight the potential of epigenetic tools to enhance crop varieties through improved yield, stress tolerance, nutritional content, and medicinal properties. Additionally, it will address the challenges and limitations associated with the application of epigenetic modifications for plant genetic improvement, providing a comprehensive understanding of both their potential and their constraints.

Mechanisms of plant epigenetic Process

Plants employ several sophisticated epigenetic mechanisms to modulate gene expression in response to environmental stressors. These strategies include DNA methylation, histone modifications, and small RNA-mediated gene silencing, each playing a unique and essential role in the regulation of gene expression and maintaining genomic integrity under stress (Abdulraheem *et al*., 2024) Figure 1.

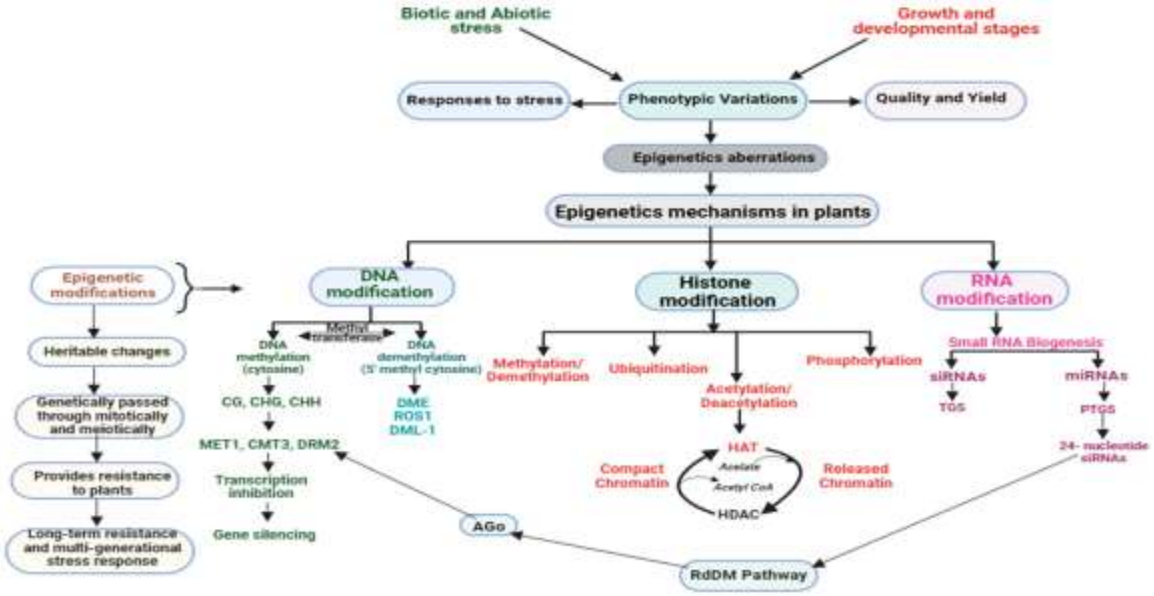

Figure 1. Epigenetic processes and mechanisms of plant adaptation to stress (Abdulraheem *et al*., 2024).

DNA methylation and demethylation

Two crucial biological processes that include the addition or removal of methyl groups (CH_3) from the DNA molecule are DNA methylation and demethylation. This may affect chromatin shape and gene expression. DNA methylation involves attaching a methyl group to the cytosine's C5 position to create 5-methylcytosine (Figure 2). Through the recruitment of proteins involved in gene repression or the inhibition of transcription factor(s) binding to DNA, DNA methylation controls the expression of genes. It governs a variety of functions including expression of a gene, genomic stability, gene imprinting, inactivation of transposable elements and its disruption can result in developmental abnormalities (Lang *et al.*, 2017; Zhang and pollin, 2018; Gallego-Bartolome, 2020).

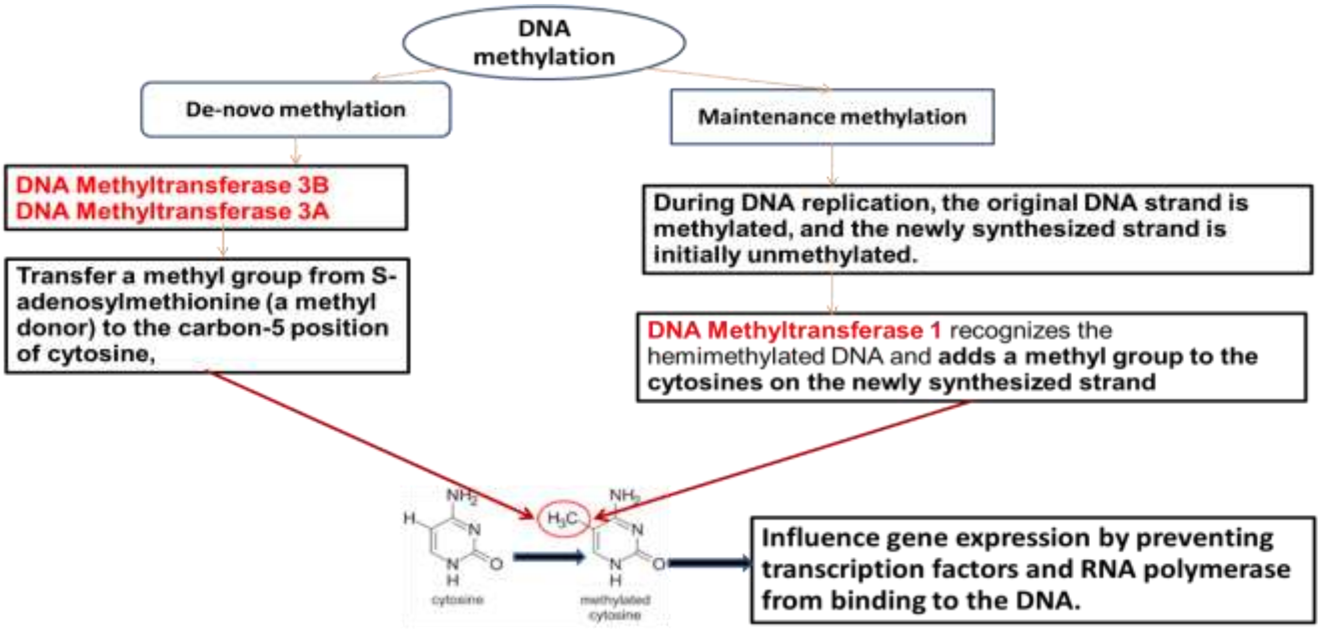

Figure 2. **Mechanisms of DNA Methylation: De-novo and Maintenance Methylation Pathways**

In contrast, DNA demethylation involves the removal of the methyl group attached to the cytosine base of DNA (Preez *et al.*, 2020). The presence of 5-hydroxymethylcytosine (5hmC) is a key intermediate step in the process of active demethylation, which can occur either passively or through active mechanisms (Lucibelli *et al.*, 2022) Figure 3.

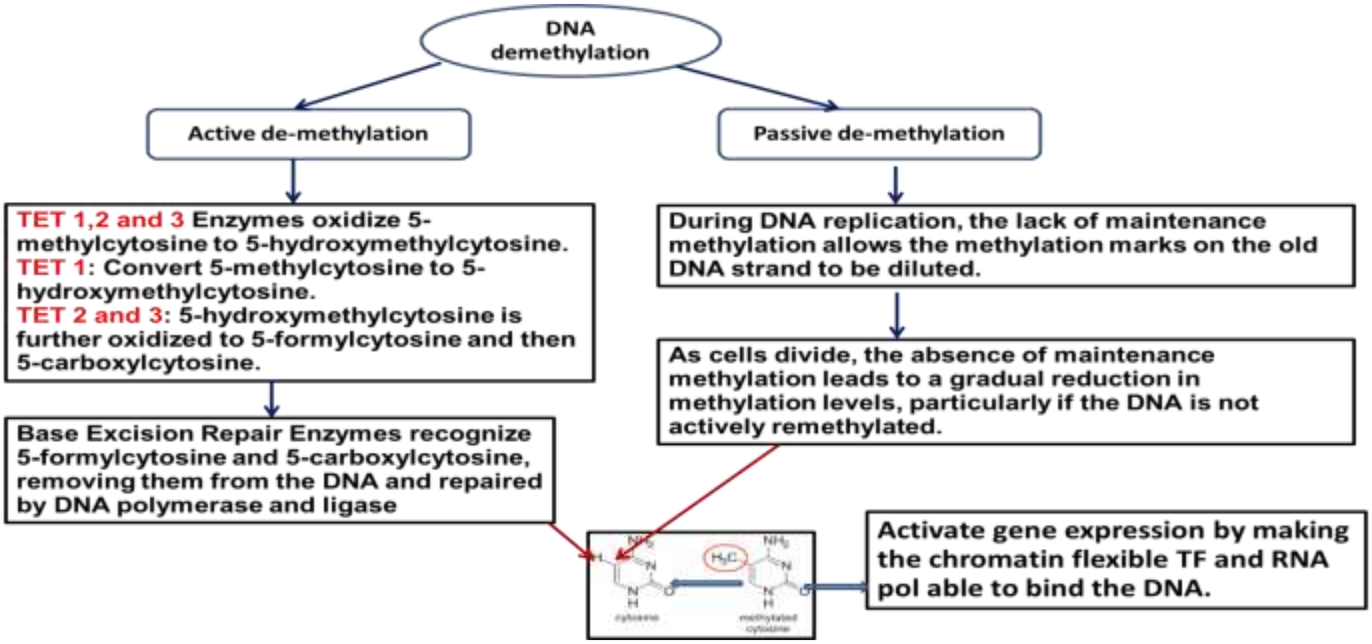

Figure 3. Mechanisms of DNA Demethylation: Active and Passive Demethylation Pathways

Typically, the methylation pattern in DNA is categorized as symmetric (CG), asymmetric (CHG), or hybrid (CHH; H = nucleotide other than G) (Figure 4). Repetitive sequences and heterochromatic transposable elements (TEs) are particularly abundant in these patterns. DNA methylation in genomic regions controlling gene expression can induce transcriptional gene silencing (TGS). The involvement of DNA methyl-readers such as SU(VAR)3-9 HOMOLOGS 1 (SUVH1) and SU(VAR)3-9 HOMOLOGS 3 (SUVH3), which are homologs of SU(VAR)3-9, in enhancing gene expression has been documented (Gale *et al*., 2018; Xiao *et al*., 2020).

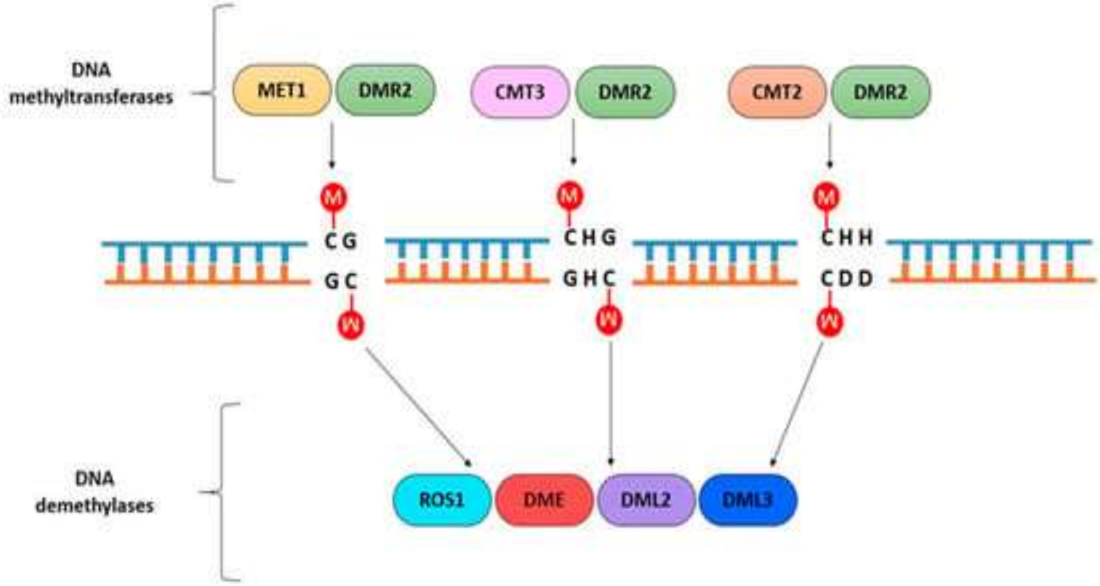

Figure 4. Specific DNA methyltransferases and demethylases mediate cytosine methylation (red circle) in different sequence contexts. CG, CHG, and CHH methylation (Lucibelli, *et al*., 2022).

When previously unmethylated cytosine residues are methylated, new methylation patterns are formed, a process known as *de novo* methylation takes place. However, during DNA replication, preexisting methylation patterns are maintained in maintenance methylation. The complex processes of DNA methylation and demethylation comprise an enormous number of enzymes and pathways (Jogam *et al*., 2022). DNA methylation and demethylation are regulated by the enzymes methyltransferases and demethylases. METHYLTRANSFERASE 1 (MET1) and CHROMOMETHYLASE 3 (CMT3) are responsible for the methylation of CG and CHG, respectively (Zhang *et al*., 2018). The *de novo* DNA methyltransferases DMNT3a (DNMT3A (DNA (cytosine-5)-methyltransferase 3 alpha)) and DMNT3b (DNMT3A (DNA (cytosine-5)-methyltransferase 3 beta) set DNA methylation patterns. Conversely, the ten-eleven translocation (TET) enzyme family mediates DNA demethylation (Lanata *et al*., 2018). Active demethylation is a sequential process in which TET enzymes first oxidize 5-methylcytosine to 5-hydroxymethylcytosine, then further oxidize it to 5-formylcytosine and 5-carboxylcytosine. These modified bases are then recognized and removed by base excision repair enzymes, which results in the final removal of the methyl group and the restoration of an unmethylated cytosine (Liu *et al*., 2023).

DNA methylation alters the chromatin structure and inhibits gene transcription because it can regulate gene expression during stress response and plant development. It controls vital traits of plants; including as disease resistance and stress from the environment. Liu *et al*. (2023) state that *de novo* DNA methylation is the result of a coordinated process that includes passive demethylation and the maintenance of methylated cytosine. Since 1975, DNA methylation has been shown to be a critical regulator of gene expression (Holliday and Pugh, 1996).

To control gene expression and maintain the stability of the plant genome, DNA methylation is necessary (Lee *et al*., 2023). The biological processes that are impacted by this 5 mC include reaction to environmental stress, transcriptional inactivity, genomic stability, and developmental control (Zhang *et al*., 2018). According to Chachar *et al* (2022), methylation and demethylation reactions both control the amount of this restrictive signal, which inhibits gene expression. X chromosome inactivation, transposable element suppression, genomic imprinting, and gene expression modulation are some of the processes that can include the active or passive modification of DNA methylation (Preez *et al*., 2020). According to Hang *et al*. (2023), DNA methylation improves crop productivity, disease resistance, and tolerance to environmental challenges. Conversely, DNA demethylation plays a role in cell differentiation, development and the reactivation of silenced genes (Preez *et al*., 2020). To ensure that gene expression and cellular function are balanced, the methylation and demethylation processes are strictly controlled.

Histone modification

Histone modifications comprise an interesting part in epigenetics (Zhang *et al*., 2021). Histone proteins act as winder around which the segment of DNA is wrapped and leads to the formation of a structural unit called as nucleosome. Nucleosomes comprise histone octamers consisting of two copies of each of the H2A, H2B, H3, and H4 histone proteins. The N-terminal tail of these histone proteins undergoes different modifications, such as acetylation, methylation, sumoylation, ubiquitination, and phosphorylation, which can change how accessible DNA is to transcription factors and other regulatory proteins (Beltran, 2022) (Figure. 5). Dynamic chemical changes to histone proteins are essential for regulating gene expression and chromatin structure. These modifications create a complex "histone code" that is a crucial epigenetic mechanism that controls many biological functions in plants (Zhang *et al*., 2021). These alterations form an active and reversible epigenetic coding that functions as an extra-genomic regulatory layer (Luo *et al*., 2021).

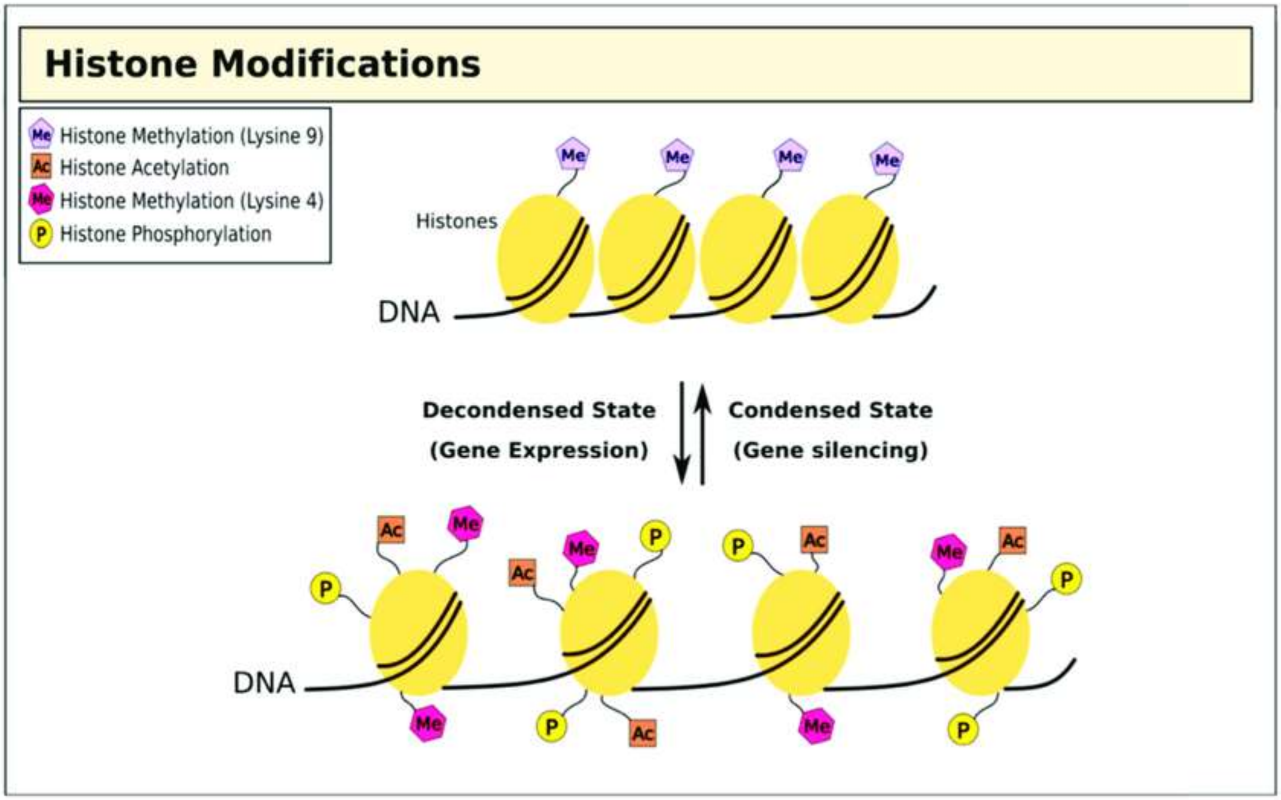

Figure 5. Histone modifications and chromatin structure: Histone methylation at lysine residues is associated with both gene expression and silencing, while acetylation is associated with repression (Akil *et al*., 2020).

Histone acetylation and deacetalyzation

Histone acetylation is defined as the addition of an acetyl group to the amine group of the histone protein's N-terminal lysine residue (Kim, 2020). Acetylation improves hydrophobicity and balances the positive charge on the histone tails (Onufriev and Schiessel, 2019). As a result, histone proteins' affinity for negatively charged DNA decreases and the chromatin state shifts from a closed to an open state (Kikuchi *et al*., 2023). This initiates the transcription process by causing RNA polymerase and transcription factors to bind to the promoter region of the gene. Research has shown a positive correlation between increased histone acetylation in the region of the transcription start site and the expression of genes (Kim, 2020). Histone acetyltransferases (HATs) facilitate histone acetylation by adding acetyl groups to lysine residues in histone globular domains and N-terminal tails, which activates gene expression. Conversely, histone deacetylases (HDACs) remove these acetyl groups, leading to transcriptional repression, chromatin condensation, and increased interaction between DNA and histones, effectively counteracting the effects of HATs (Li *et al*., 2018; Kumar *et al*., 2022) (Figure 6). Both HATs and HDACs primarily target lysine residues such as H3K9, H3K14, H3K36, H4K5, H4K8, H4K12, and H4K16 (Li *et al*., 2018). Proteins containing bromodomains recognize these acetylated lysine residues on histones, thereby influencing gene expression (Wang *et al*., 2020) (Figure 6).

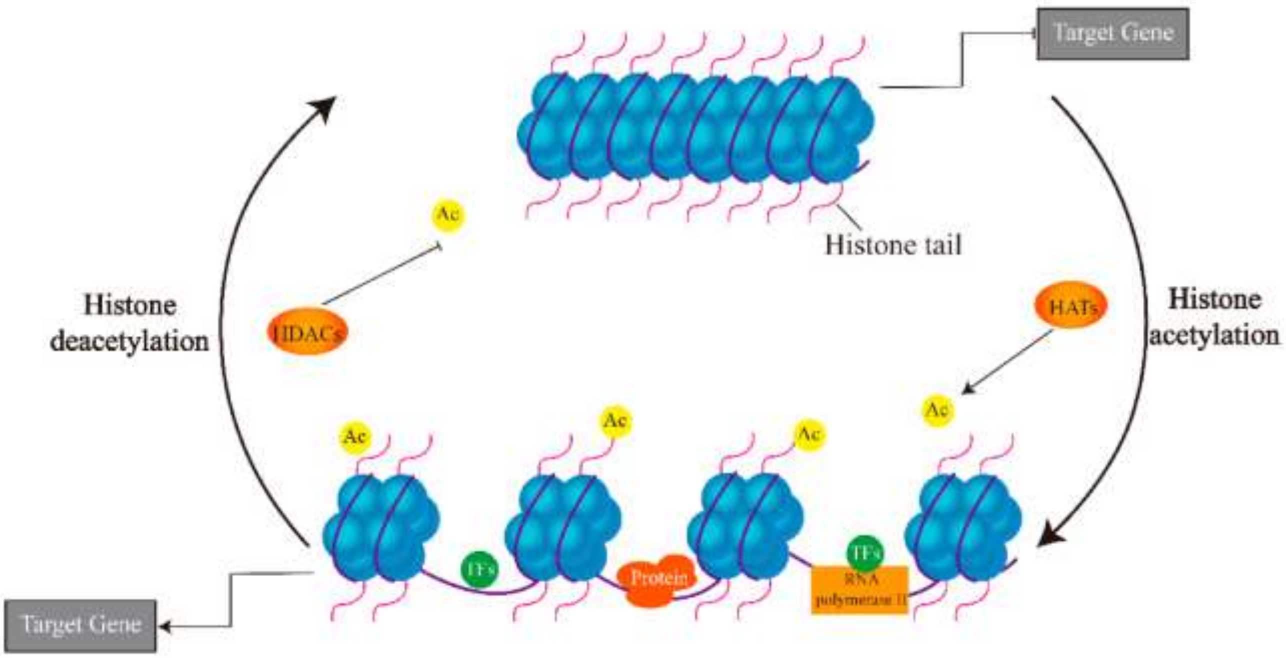

Figure 6. Regulation of histone acetylation by HATs and HDACs (Li *et al.*, 2021).

Histone methylation and demethylation

Histone methylation required for biological processes like transcriptional control and the formation of heterochromatin leads to retention of the charge on amino acids and does not affect electrostatic properties of histones (Ueda and Seki, 2020). The primary sites of modification in H3 and H4 histones are arginine (R) and lysine (K) residues. This leads to a change in gene expression that can have both repressive and activating effects. This alteration has a multitude of functions in controlling the expression of genes and can be mono-, di-, or trimethylated. Trimethylation of Lys 4 (H3K4me3) raises gene transcription in *A. thaliana*, but trimethylation of Lys 27 (H3K27me3) decreases gene expression (Zhang *et al*., 2021).

Histone methylation is the transfer from S-adenosyl-L-methionine of one to three methyl groups, to lysine or arginine residues of histone proteins. Histone methylation, catalyzed by histone methyltransferases (HMTs), involves the methylation of lysine residues on histones H3 and H4, which can either activate or repress transcription depending on the specific methylation site, while arginine methylation is associated solely with transcriptional activation. These methylation processes are facilitated by enzymes known as lysine methyltransferases (Liu *et al*., 2022). Functional diversity to the individual methylation sites is provided by the fact that lysine can be mono-, di- or trimethylated. For instance, both mono- and tri-methylation of K4 on histone H3 cause transcriptional activation, but the former occurs at transcriptional enhancers and the latter occurs at gene promoters. The tri-methylation of K9 and K27 on histone H3 are signals for the repression of transcription. Trimethylation of K27 mainly occurs at promoters in gene-rich chromosomal regions and controls developmental regulators in the stem cells of embryos, while trimethylation of K9 usually occurs in gene poor regions such as telomeres or satellite repeats (Zhang *et al*., 2021).

Histone demethylation, as opposed to methylation, is the process of removing methyl groups from histone residues and mediated by the enzyme Histone demethylases (HDMs), which remove methyl marks to enable regulation of gene expression, are in charge of this dynamic process. On the other hand, arginine demethylation is a very new field of study, and recent work indicates that proteins such as arginine demethylase 1 (AtRDM1) may be involved (Li *et al*., 2016) (Figure 7).

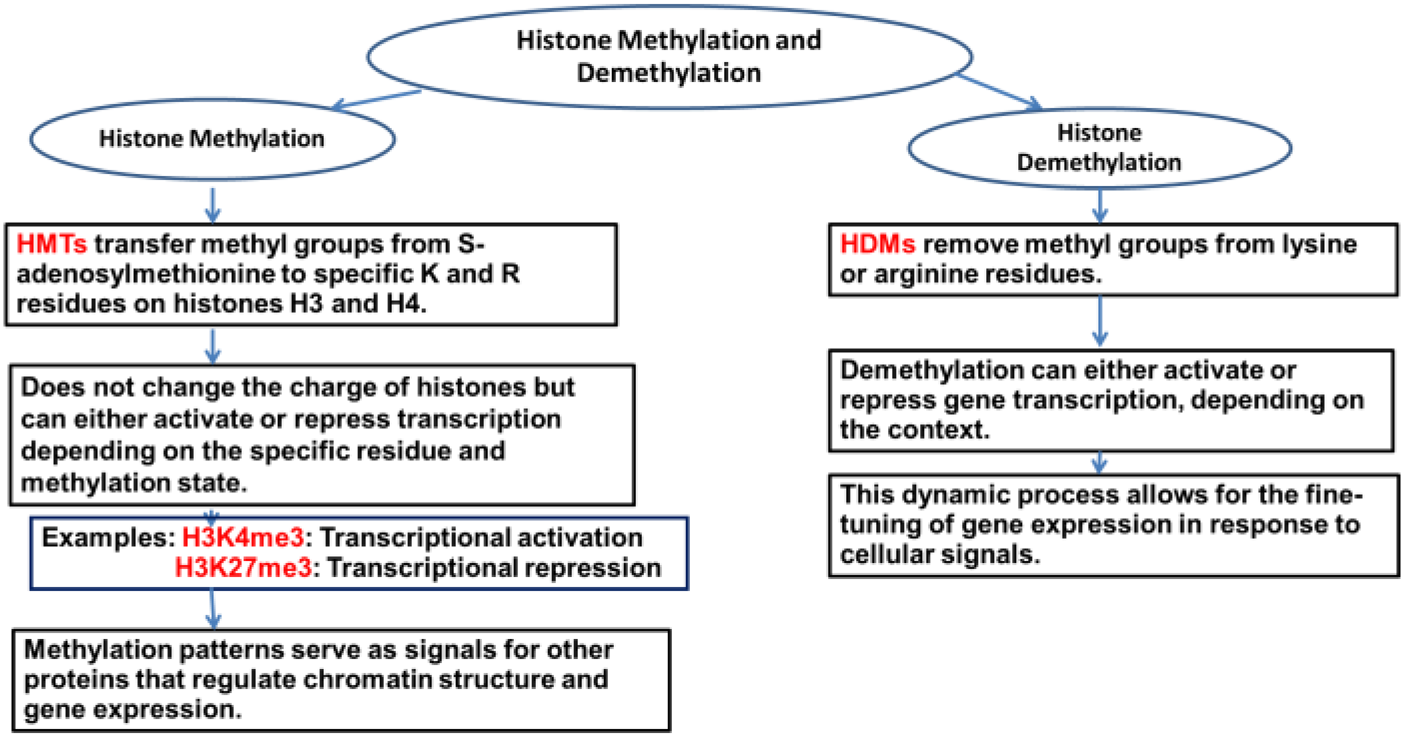

Figure 7. Mechanisms of Histone methylation and demetylation.

Histone Phosphorylation and de phosphorylation

The process of adding phosphate groups to a molecule is phosphorylation, particular to serine, threonine, or tyrosine residues on histone proteins is known as histone phosphorylation. Numerous biological functions, such as DNA repair, gene transcription, and reaction to external stimuli are linked to this alteration. Protein phosphatases aid in the removal of phosphate groups, whereas protein kinases are responsible for their addition (Figure 8). These enzymes help to maintain the chromatin structure's flexibility by dynamically controlling histone phosphorylation (Zhang *et al.*, 2019). Both transcriptional inhibition and activation are linked to histone phosphorylation at certain sites. The recruitment of transcriptional activators or repressors by phosphorylation events can affect the transcriptional machinery's accessibility to target genes (Wang *et al.*, 2019).

The way cells react to their damaged DNA is largely dependent on histone phosphorylation: Maintaining the integrity of the genome is made possible by phosphorylation events, which draw repair proteins to damaged areas (Zhang *et al.*, 2019). Cell Cycle Control: Specific phosphorylation processes take place during several phases of the cell cycle, and histone phosphorylation is closely associated with this cycle. Appropriate cell cycle progression and division are ensured by this regulatory mechanism (Liu *et al.*, 2022) and responses to environmental stress: Numerous environmental stresses are known to affect plants, and the activation of genes that respond to stress is linked to histone phosphorylation. As a component of the signaling cascade, phosphorylation events help plants adjust to shifting environmental conditions (Liu and Zhang, 2018).

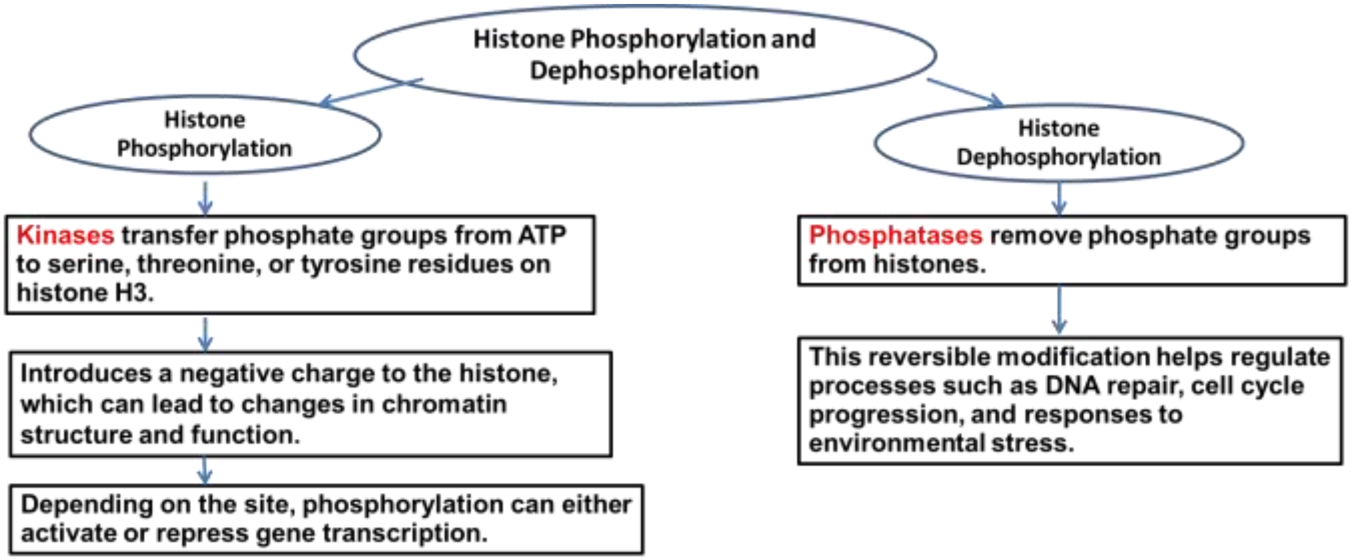

Figure 8. Mechanisms of Histone phosphorylation and dephosphorization

Histone Ubiquitination

The process of histone ubiquitination entails ubiquitin molecules attaching themselves covalently to lysine residues on histone proteins. This alteration can take the form of poly-, multi-, or mono-ubiquitination, each of which has unique functional implications for the dynamics of chromatin and the regulation of gene expression (Hou *et al*., 2020). The dynamic equilibrium of ubiquitination on histones is maintained by ubiquitin ligases, which add ubiquitin to histones, and deubiquitinases, which remove ubiquitin (Dong *et al*., 2020). Histone ubiquitination modifies the chromatin structure, which in turn affects gene expression. Depending on the specific lysine residues targeted and the genomic area background, it can either stimulate or repress transcription (Hou *et al*., 2020).

Histone ubiquitination is a critical player in plant responses to environmental stresses. It regulates the expression of stress-responsive genes, enabling plants to adapt to challenging conditions, genomic stability and DNA repair, that is, it contributes to maintaining genomic stability by participating in DNA repair processes. It facilitates the recruitment of repair factors to damaged DNA sites (Chen *et al*., 2017) and involved in the silencing of transposable elements, contributing to genome defense and stability (Zhang *et al*., 2019).

Histone Biotinylation

Histone biotinylation, a post-translational modification involving the covalent attachment of biotin (vitamin B7) to histone proteins, has garnered increasing interest for its potential role in regulating chromatin dynamics and gene expression. This modification is catalyzed by biotin ligases, which transfer biotin onto specific lysine residues on histones, thereby modulating chromatin structure and function (Geng *et al*., 2018). The reversible nature of histone modifications suggests the existence of debiotinylases, enzymes that remove biotin from histones, although these have yet to be clearly identified and characterized (Huo *et al*., 2018).

Recent studies highlight the importance of histone biotinylation in plants, where it appears to influence various aspects of chromatin dynamics and gene regulation. For instance, research has shown that biotinylation of histones can affect nucleosome stability, chromatin compaction, and the recruitment of other regulatory proteins, thereby playing a crucial role in the epigenetic regulation of gene expression. This emerging understanding positions histone biotinylation as a significant but underexplored mechanism in the epigenetic landscape of plants (Rahikainen *et al*., 2019).

The expanding research into histone biotinylation underscores its potential as a novel target for manipulating plant growth and development. By modulating biotinylation levels, it may be possible to influence gene expression patterns, offering new strategies for crop improvement and stress resilience. As our understanding of histone biotinylation deepens, it is likely to reveal further insights into its regulatory roles and interactions within the broader epigenetic framework.

Histone Sumoylation

Histone sumoylation is catalyzed by a series of enzymes, including E1 activating enzymes SUMO Activating Enzyme 1 and SUMO Activating Enzyme 2 (SAE1/SAE2), E2 conjugating enzyme ubiquitin-conjugating enzyme (Ubc9), and E3 ligases Protein Inhibitors of Activated STATs (PIAS proteins). These enzymes collaborate to transfer the Small Ubiquitin-like Modifier (SUMO) moiety to specific lysine residues on histones (Mazur *et al.,* 2020). Particular lysine residues on histone tails act as substrates during sumoylation, exhibiting target selectivity. This modification accuracy is aided by recognition motifs and interactions with SUMO ligases (Mazur *et al.,* 2020).

Conversely, deSUMOylation is mediated by SENP (SUMO/sentrin-specific protease) enzymes, which play a critical role in reversing the sumoylation process. SENP enzymes are responsible for the precise removal of SUMO (Small Ubiquitin-like Modifier) moieties from target proteins, including histones. This process is essential for maintaining the dynamic balance of sumoylation and desumoylation within the cell. By cleaving the isopeptide bond between SUMO and the lysine residues on target proteins, SENPs ensure the modulation of protein function, stability, localization, and interactions. The activity of SENP enzymes is crucial for various cellular processes, including the regulation of chromatin structure and gene expression, DNA repair, and signal transduction pathways. This reversible modification system allows cells to respond rapidly to changing conditions and to fine-tune protein functions as needed (Cheng *et al.*, 2019).

The chromatin architecture is influenced by histone sumoylation, which affects gene transcription. Depending on the situation and particular histone residues altered, it can function as an activator or a transcriptional repressor (Castro *et al.*, 2018).

In plants, histone modification has been found to play a significant role in stress response when the stressors are biotic or abiotic. Acetylation is linked to the activation of stress-responsive genes, implying a direct relationship connecting histone changes and the plant's ability to adapt to water deprivation. Similarly, salt stress has been found to induce changes in histone methylation patterns, affecting the expression of genes involved in ion homeostasis and osmotic regulation (Zhao *et al.*, 2019).

In general, histone modifications have been shown to play a role in various aspects of plant development, including flowering time, seed germination, and stress response (Luo *et al.*, 2023). By manipulating histone modifications, researchers can alter gene expression patterns, improve plant traits such as yield, and stress tolerance (Chachar *et al.*, 2022).

Non-Coding RNA in Plant Epigenetic Regulation

RNA exists in two primary categories: coding and non-coding. While coding RNAs are translated into proteins, non-coding RNAs (ncRNAs) function independently of translation, serving essential roles in cellular processes. Among ncRNAs, certain types—such as long non-coding RNAs (lncRNAs), small interfering RNAs (siRNAs), and microRNAs (miRNAs)—are key regulators of gene expression and are instrumental in epigenetic modifications. These specialized ncRNAs employ unique mechanisms to modulate gene expression in plants, highlighting their importance in the intricate regulation of genetic activity (Figure. 9).

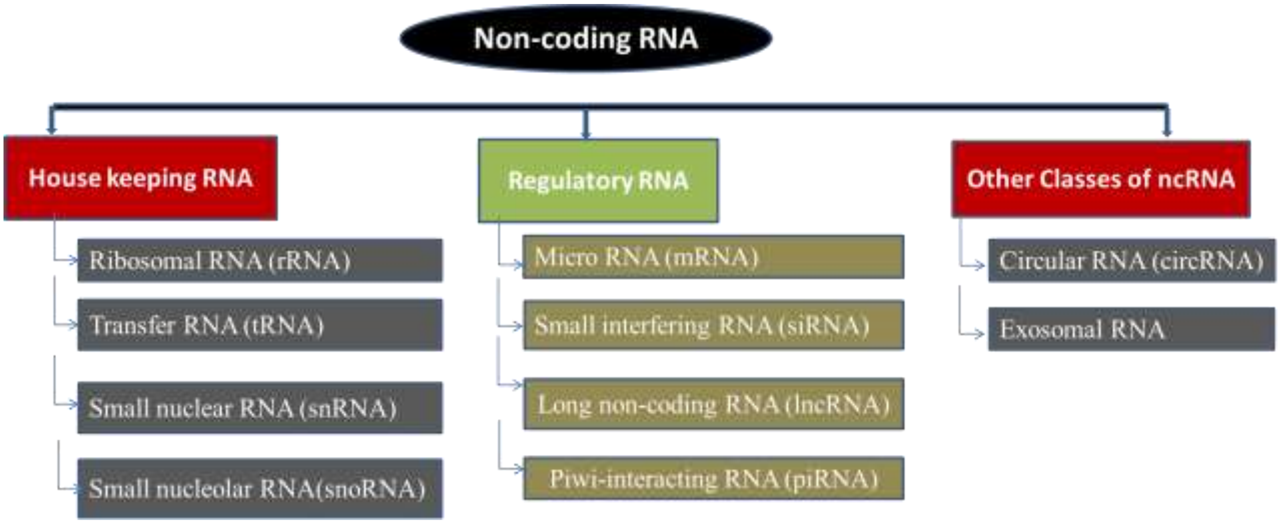

Figure 9. Classification of regulatory RNA

In the field of plant epigenetics, small RNA-mediated modification has emerged as a relatively new and growing area of study (Qi *et al.*, 2023). Short ncRNAs such as siRNAs and miRNAs are known to target specific genes, influencing gene expression through modifications to histones and DNA methylation patterns. This process allows plants to adapt their gene expression profiles in response to various environmental stresses, such as disease and adverse climatic conditions. For instance, small RNA-mediated epigenetic changes are linked to key traits in plants, including stress response, disease resistance, and adaptation to environmental challenges (Kumari *et al.*, 2017)

The potential for manipulating these epigenetic modifications offers exciting avenues for plant improvement. By altering small RNA pathways, researchers could enhance desirable plant characteristics such as yield, stress tolerance, and disease resistance, paving the way for more resilient and productive crops. This area of research holds considerable promise for sustainable crop production, aligning with goals of increased agricultural productivity and resilience (Huang and Jin, 2022).

MicroRNAs (miRNAs)

MicroRNAs have an average length of around 21 nucleotides. In eukaryotes, they play a crucial role in post-transcriptional gene regulation. Since their discovery in the early 1990s, miRNAs have come to be understood as essential parts of the cellular machinery, coordinating complex networks of gene expression to regulate development, preserve homeostasis, and react to external stimuli (Lin *et al.*, 2024).

The miRNA biogenesis is a complex process that starts in the nucleus and ends in the cytoplasm. Primor-miRNAs, the first transcripts produced by miRNA genes, are usually transcribed by RNA polymerase II. The enzyme Drosha then cleaves these pri-miRNAs to produce precursor miRNAs, or pre-miRNAs. Pre-miRNAs are exported to the cytoplasm, where they are further processed by Dicer to produce mature miRNAs. The RNA-induced silencing complex (RISC) is guided to target mRNAs by the mature miRNAs that are placed onto it (Huang and Jin, 2022).

The 3' untranslated region (UTR) of target mRNAs is where miRNAs mostly bind to achieve their regulatory effects, while interactions with other areas, such as the coding sequence, have also been noted. Base-pairing interactions, which usually entail partial complementarity between the miRNA and the mRNA, are how the miRNA directs the RISC to its mRNA target (Figure 10).The regulatory result is influenced by the degree of complementarity (Migliorini *et al.*, 2023).

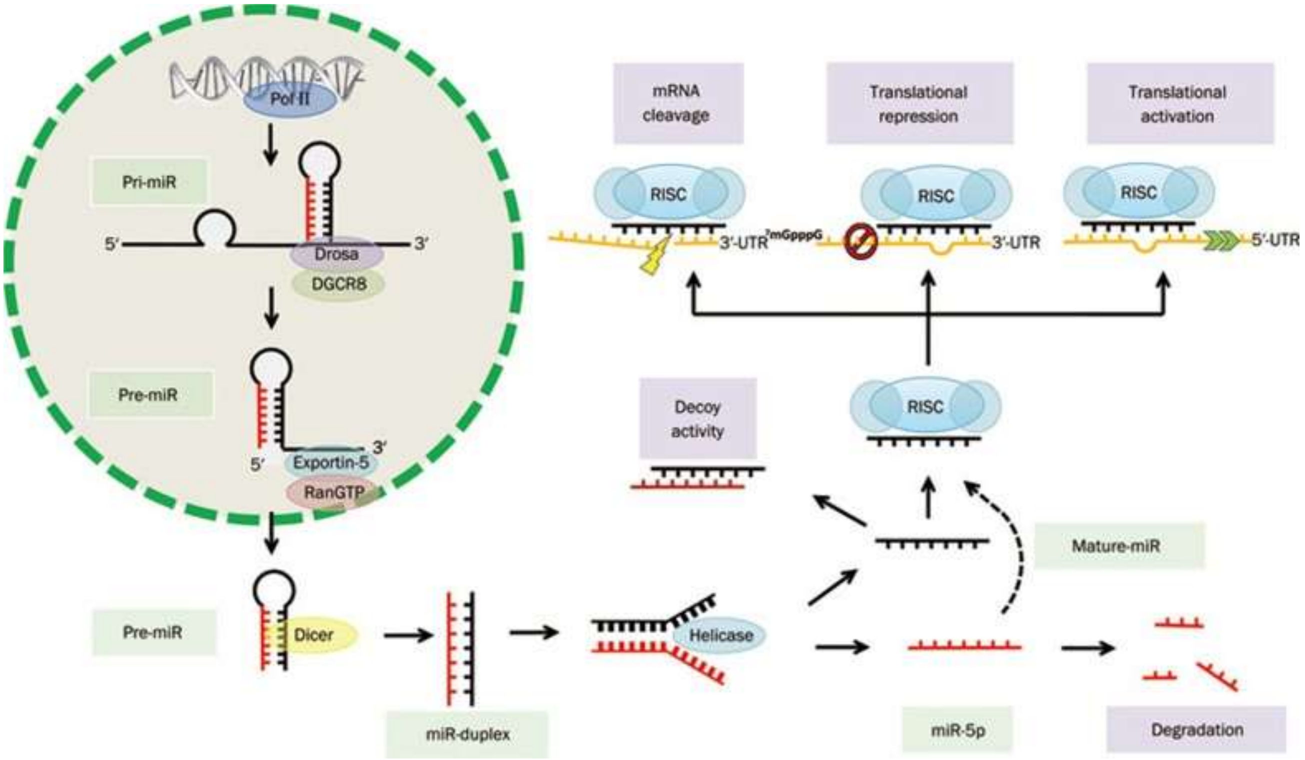

Figure 10. The mechanism of microRNA biogenesis and regulation of gene expression (Li and Yang, ,2013).

Small Interfering RNAs (siRNAs):

RNA interference (RNAi) is a potent and conserved mechanism that regulates gene expression and provides defense against viruses and transposons. Small interfering RNAs (siRNAs) are double-stranded non-coding RNAs that are essential to the process. siRNAs, which are mainly 21–23 nucleotides long, serve as instructions for the RNA-induced silencing complex (RISC), allowing corresponding mRNA sequences to be recognized specifically and then modulated (Suleiman *et al*., 2024).

Recognition and cleavage of lengthy double-stranded RNA (dsRNA) precursors usually precedes the production of siRNAs. These precursors can come from a number of different places, such as endogenous cellular processes, transposon activity, and viral infections. An essential step in the conversion of dsRNA into siRNAs is taken by the RNase III family enzyme Dicer. The siRNA duplexes with distinctive 2-nucleotide overhangs at their 3' ends are created when the dicer splits the dsRNA into small fragments (Hung and Slotkin, 2021).

siRNAs primarily induce gene silencing through two main mechanisms: mRNA degradation and translational repression. In cases of perfect or near-perfect complementarity between the siRNA and its target mRNA, the RISC complex induces cleavage and subsequent degradation of the mRNA by cellular exonucleases. On the other hand, imperfect base pairing may lead to translational repression, where the RISC interferes with ribosome binding and protein synthesis without causing mRNA degradation (Yu *et al*., 2023).

Long Non-Coding RNAs (lncRNAs)

Long non-coding RNAs (lncRNAs) are a flexible type of non-coding RNA molecules that are distinguished by their length typically more than 200 nucleotides and their inability to code for proteins. RNA was once thought to be only a means of facilitating the creation of proteins. However, recent research has shown that lncRNAs play a major role in the complex regulation of multiple cellular processes (Mattick *et al*., 2023).

lncRNAs are essential for controlling the structure and function of chromatin. They can serve as scaffolds, directing chromatin-modifying complexes to particular genomic loci, affecting DNA methylation patterns and histone modifications, and taking role in transcriptional control of gene expression. They have the ability to affect the start, elongation, or end of transcription through interactions with transcription factors, RNA polymerase, and other regulatory proteins (Mattick *et al*., 2023).

Circular RNAs (circRNAs)

Circular RNAs (circRNAs) are a particular type of non-coding RNAs that are distinguished by their covalently closed-loop structure without free ends, which confers increased stability and resistance to exonucleases. Circular RNAs are also thought to be rare because they lack the traditional linear 5' to 3' ends, which are thought to be the result of mis-splicing. However, recent extensive research has shown that circRNAs are widespread and play a variety of regulatory roles in various cellular processes (Liu *et al*., 2023).

circRNAs are mostly produced by a process called back splicing, which is the joining of a downstream splice donor site to an upstream splice acceptor site. This process creates a circular structure from a precursor mRNA. Developments in high-throughput sequencing technologies have shown that circRNAs are widely distributed in different tissues, organisms, and cell types. They also show a great deal of diversity in terms of size, sequence, and abundance (Liu, *et al*., 2023).

As microRNA (miRNA) sponges, circRNAs can trap miRNAs and stop them from attaching to the mRNAs that they are intended to bind to. Additionally, they have the ability to interact with RNA-binding proteins (RBPs), modifying cellular processes and their activity (Chen, 2016).

Chromatin Remodeling

Chromatin remodeling complexes are crucial for the epigenetic regulation of gene expression in plants, especially during times of stress (Tonosaki *et al*., 2022). According to Pandey *et al*. (2016), these complexes are responsible for altering the structure of chromatin, which affects DNA accessibility to transcriptional machinery and regulatory proteins. Chromatin remodeling complexes are involved in the modification of stress-responsive gene expression in the context of plant stress, including abiotic and biotic challenges. This has an impact on the plant's ability to adapt and survive in harsh environments (Kim, 2019).

In order to ensure their survival and regulate gene expression, plants use chromatin-remodeling complexes to reprogrammed gene expression patterns in response to stress. These complexes facilitate structural alterations that either activate or repress stress-responsive genes (Wang *et al*., 2020). Plant stress responses involve chromatin-remodeling complexes that modify gene expression through the ejection, sliding, or modification of nucleosome composition using ATP hydrolysis energy (He *et al*., 2023).

Histone-modifying enzymes can either stimulate or inhibit gene transcription by applying posttranslational modifications to histone proteins. Because certain chromatin structural changes result from stress signals, making DNA accessible, the chromatin arrangement prevents transcription factors, polymerases, and other nuclear proteins from accessing DNA when it is severely condensed (Bhadouriya *et al*., 2020). Fast and reversible changes in gene expression in response to plant stress are made possible by chromatin remodeling complexes, which are essential for epigenetic regulation (Pecinka *et al*., 2020).

Plants are able to adapt to different stressors in an efficient manner because to this dynamic control, all without affecting the genetic code at all. Chromatin remodelling complexes work with other epigenetic mechanisms, such as DM and small RNA-mediated silencing pathways, to coordinate comprehensive responses to stress. Plant resilience, adaptation, and stress response are the result of cross talk across these several tiers of epigenetic regulation. The activity and selectivity of chromatin remodeling complexes can be influenced by environmental cues, according to recent research, demonstrating a clear connection between outside stimuli and epigenetic modifications that affect the expression of genes that respond to stress (Kim, 2020).

Methods to modify the plant epigenome

Applying chemicals, biotic and abiotic stresses, tissue culture, mutagenesis, grafting, or molecular RNA-based techniques like RNAi and CRISPR are among the techniques currently employed in the lab to alter the plant epigenome in order to produce novel phenotypes (Dalakouras and Vlachostergios, 2021).

Numerous treatments have an impact on the epigenome of plants. According to Nowicka *et al*. (2020), the non-methylable cytidine analogues 5-azacytidine, 5-aza-2′-deoxycytidine (decitabine), and zebularine are the most often used inhibitors of DNA methylation. The mode of action of azacytidine has been well-documented. When applied to seedlings, it is taken up by plant cells and incorporated into DNA, replacing cytosine with a non-methylable analogue during each cycle of DNA replication. The DNA methyltransferases identify azacytosine–guanine dinucleotides as a natural substrate for nucleophilically attacking to start the methylation reaction. The enzyme and the carbon–6 atom of the cytosine ring form a covalent connection. The bond is broken by β-elimination through the carbon-5 atom; however, azacytosine, which substitute's nitrogen for carbon-5, blocks the reaction. The enzyme's ability to operate as a methyltransferase is so inhibited and it stays covalently attached to DNA. Furthermore, covalent protein adduction impairs DNA function and initiates DNA damage signaling, which causes imprisoned DNA methyltransferases to degrade. Consequently, during DNA replication, methylation markers are erased. POL II inhibitors, including α-amanitin and actinomycin D, can also be employed as demethylating agents in addition to nucleotide analogues (Thieme *et al.*, 2017).

DNA hypomethylation has also been induced by other pharmacological agents with mechanisms that are not fully understood, such as genistein, a phytoestrogen found in soybeans. While the precise mechanism of genistein-induced DNA hypomethylation remains unclear, studies suggest it may involve the inhibition of DNA methyltransferases (DNMTs) or the activation of DNA demethylation pathways (Chen *et al.*, 2010). Other pharmacological agents, such as 5-azacytidine and decitabine, are well-known DNMT inhibitors that lead to DNA hypomethylation by incorporating into DNA and trapping DNMTs during the methylation process (Christman, 2002).

In addition to DNA methylation, certain substances can also influence histone modifications. For instance, trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor, prevents the removal of acetyl groups from histone tails. This inhibition leads to an open chromatin structure, thereby facilitating increased gene expression (Nowicka *et al.*, 2020). By targeting HDACs, TSA indirectly interacts with DNA methylation pathways, highlighting the interconnected nature of epigenetic regulation. Additionally, sulfamethazine and 3-deazaneplanocin A (DZNep) treatments may suppress histone methylation by blocking the activity of S-adenosyl-homocysteine (SAH) hydrolase, an enzyme that converts SAH to homocysteine and adenosine (Nowicka *et al.*, 2020). This inhibition by DZNep increases the level of SAH in cells, disrupting the metabolism of methionine and reducing the level of S-adenosyl-methionine, the methyl donor in enzymatic methylation reactions (Miranda *et al.*, 2009). Some examples of methods of epigenome modification in different plant are listed below (Table 1).

Table 1. Methods for epigenome modification in various plants.

Plant	**Method**	**Outcome**	**Reference**
Arabidopsis	3-Deazaneplanocin A	DNA hypomethylation	Nowicka *et al.*, 2020
Arabidopsis	α-Amanitin	DNA hypomethylation	Thieme *et al.*, 2017
Hordeum vulgare	Drought stress	DNA hypermethylation	Surdonja *et al.*, 2017
Vicia faba	Drought stress	DNA hypomethylation	Abid *et al.* 2017
	Heat stress	Histone methylation and acetylation	Hou *et al.*, 2019
Arabidopsis	N-acetylglutamic acid	Histone acetylation	Hirakawa *et al.*, 2024
Nicotiana benthamiana	RNAi/dsRNA spraying	Gene-specificDNA hypermethylation	Dalakouras and Ganopoulos, 2021
Nicotiana benthamiana	RNAi/IR-transgene	Gene-specific DNA hypermethylation	Dadami *et al.* 2014
Hevea brasiliensis	Grafting	DNA methylation alterations in the scion	Uthup *et al.*, 2018
Cucumis sativus, Cucumis melo, Cucurbita pepo	Grafting	DNA hypermethylation in the scion	Avramidou *et al.*, 2015
Elaeis guineensis	Tissue culture	Histone hypoacetylation	Yaacob *et al.*, 2013
Elaeis guineensis	Tissue culture	DNA hypomethylation	Ong-Abdullah *et al.*, 2015

The Role of epigenetics in plant growth, development, and reproduction

Epigenetic mechanisms play a crucial role in plant growth and development. Epigenetic marks are acquired during plant development and can be inherited by subsequent generations. By regulating gene expression patterns, epigenetic mechanisms help fine-tune plant growth and development, allowing for adaptive responses to changing environmental conditions (Zhang *et al*., 2007). These processes are essential for proper plant function, and disruptions to epigenetic regulation can lead to developmental abnormalities and reduced fitness (Yamaguchi, 2022). Epigenetic regulation also plays a significant role in the control of flowering time in plants. The FLC locus is one example of how epigenetic mechanisms can reprogram gene expression and control flowering time in vernalization-sensitive plants (Gehring, 2019). Epigenetic changes can lead to transposon silencing, paramutation, and genomic imprinting, all of which are involved in regulating flowering time by controlling the timing of flowering, epigenetic regulation can affect plant reproduction and ultimately, population fitness (Gehring, 2019).

Epigenetic control of reproduction in plants is complex and varies depending on the mode of reproduction. In sexually reproducing plants, epigenetic variability can have adaptive significance, as it allows for greater genetic diversity and the potential for rapid adaptation to changing environmental conditions (Zhang *et al*., 2007). In asexual plants, epigenetic regulation plays a different role, as it can maintain genetic stability and prevent the accumulation of harmful mutations (Brukhin and Albertini, 2021). Epigenetic regulation is also involved in male reproduction in plants, with recent research highlighting the role of chromatin remodeling and histone modifications in pollen development and function. Overall, the study of epigenetics in plant growth, development, and reproduction is a rapidly evolving field, with new discoveries shedding light on the complex interplay between genetic and epigenetic regulation (Han *et al*., 2019).

Applications of epigenetic modification for plant improvement

The epigenetic modifications of DNA methylation, histone modifications, and the expression of ncRNAs have important biological, physiological, and molecular consequences for plants. Today, epigenetic phenotypes are being explained by discoveries such as activation, excision, and translocation of TEs, allelic interactions, transgene silencing, and epialleles (Martienssen and Colot, 2001).

Recent studies on epiRIL in Arabidopsis demonstrate that epigenetics of QTL can explain the heritability of the complex traits. Through epigenetic modification mechanisms, crop varieties can be improved in terms of yield and quality by modulating plant development and physiological responses to environmental conditions (Sun *et al*., 2021). Plant traits can be enhanced by epigenetic diversity in a natural, genetic, and chemical manner (Kakoulidou *et al*., 2021). Agronomic traits are often quantitative and under intricate genetic control, which hinders crop improvement. In spite of these limitations, epigenetic tools offer a promising approach (Gupta and Salgotra, 2022). Through epigenetic modifications, crops can be developed to produce higher yields and better quality, which can contribute to global food security.

Epigenetic regulation is a crucial mechanism for plants to respond and adapt to stress, both biotic and abiotic. Several studies have investigated the role of DNA methylation, non-coding RNAs, and histone modifications in regulating stress-responsive genes, which can help to understand plants' ability to adapt to fluctuating environmental conditions. By studying and manipulating these epigenetic mechanisms, plant breeders can develop crops with improved resistance to biotic and abiotic stress, which can lead to more stable and sustainable agricultural systems.

In addition to increasing productivity and resilience to stress, epigenetic changes can improve a plant's nutritional content and therapeutic qualities (Tonosaki *et al*., 2022). Enhancement of growth and development, phenotypic plasticity, and flowering under stress are all possible through genetic and epigenetic reprogramming (Kumari *et al*., 2022). Recent data suggests that changes in gene expression that may be connected to the manufacture of secondary metabolites including flavonoids, alkaloids, and terpenoids can be linked to epigenetic alteration (Wu *et al*., 2023). Due to their substantial nutritional and therapeutic benefits, these metabolites can help create new plant-based medications and functional meals by increasing their production through epigenetic alteration (Duarte *et al*., 2023).

The high-resolution epigenome profiling can be used to understand the molecular mechanisms of epigenetic inheritance and identify potential targets for alteration, the complexity of epigenetic regulation and its interactions with genetic variables make applying epigenetics to crop improvement challenging (Gupta and Salgotra, 2022). Although much remains to be discovered regarding the use of epigenetics in crop growth and breeding, it is clear that epigenetic variety can serve as a valuable source of phenotypic variation (Varotto *et al*., 2020). But, further study in this area might result in crops that are more tolerant of environmental challenges and could contribute to meeting the world's growing food need.

Application of epigenetics in abiotic stress

Low water and nutrient availability, high temperatures and light levels, and soil properties like salinity and the presence of heavy metals are some of the environmental stressors that plants constantly face. Plants have developed genetic and epigenetic defence to withstand single or multiple shocks and their interactions in response to climate change and the resulting increase in environmental unpredictability (Zhang et al., 2007). Therefore, it is crucial to understand, the genetic and epigenetic foundations of crop responses to environmental changes. Consequently, Brzezinka *et al*. (2016) used priming by heat stress as a model to isolate the memory of environmental stress in Arabidopsis and identify genes that are only required for heat stress memory but not for the initial responses to heat.

FORGETTER1 (FGT1) gene, which binds directly to a class of heat-inducible genes, was identified (Kim *et al*., 2006). The same gene ensures that heat-inducible genes are constantly accessible and active by changing the packing of the DNA containing these genes their findings may lead to new approaches in crop breeding programs for enhancing resistance to abiotic stress, as knowledge of the stability and hereditary features of epigenetic marks and epigenetic regulatory systems is crucial for breeding applications (Gallusci *et al*., 2017). The aforementioned study has established a strong foundation for a deeper comprehension of the various mechanisms behind variation that impact the productivity of plants and crops.

Tolerance to drought/logging

A complex interplay of physiological, metabolic, and genetic factors gives plants the resilience to environmental stresses such as drought. The ability of plants to absorb nutrients, get water, and maintain their structural integrity can all be severely hampered by drought and logging. Reduced cell turgor pressure is the outcome of higher osmotic pressure in drought-stricken plants (Mittler. 2006). According to Saktiyono *et al*. (2022), a plant may sustain harm or perhaps perish if the drought persists until it starts to wilt permanently. One important and still-evolving area of plant biology research is drought stress. There are several ways that drought impacts a plant's phenotype, including morphological, physiological, cellular and molecular.

Plants from Arabidopsis and *Zea mays* L. that underwent dehydration stress were better able to retain water in subsequent generations or when they underwent stress later on, according to Song *et al*. (2020). Many studies have demonstrated that recurrent stress, often known as priming, helps plants better adapt to future difficulties. During drought stress, plants' epigenetic makeup is dynamically changed, and these modifications have a significant impact on how plants react to environmental cues, for instance, identified ZmHDT103, which encodes a histone deacetylase, using homologous sequence comparison. They discovered that the treatment of maize seedlings with polyethylene glycol (PEG) affects the expression of ZmHDT103 (Kim *et al*., 2006). Similar examples are given in Table 2.

Tolerance to Salt

High salt deposition in the soil has a substantial effect on plant species richness and distribution, making it another important agricultural production barrier that affects 20% of the world's farmed land (Ventouris *et al*., 2020). A genomic investigation of phenotypically diverse rice lines to investigate methylation changes under strain demonstrated that hypomethylation induced by salt stress is linked to altered expression of DNA demethylases. The phenotypic heterogeneity associated with salinity tolerance may be influenced by epigenetic modulators (Ferreira *et al*., 2015).

Ferreira *et al*. (2015) claim that epigenetic changes alter the expression of transcription factors and together impact genes that are triggered by stress. Two *B. napus* cultivars, one salt-tolerant (*cv. Exagone*) and the other salt-sensitive (*cv. Toccata*), demonstrated a rise in total DNA methylation in cv. Toccata and a fall in *cv. Exagone* when subjected to salt stress conditions (Song *et al*., 2012). A putative short RNA target area in Arabidopsis was discovered to be heavily methylated, approximately 2.6 kb upstream of HKT1. The RdDM mutant rdr2 exhibited increased

HKT1 expression and a reduced DNA methylation level in this area, indicating that RdDM negatively influences the expression of the AtHKT1 gene. Parallel guiding activity was also seen in wheat (Kumar *et al.*, 2017).

Tolerance to Heat

Heat stress is one of the primary environmental factors that seriously threaten food security as global warming grows. Extreme temperatures have been shown to have an impact on plant growth and development, including crop yield and nutritional value, in tropical regions and at high elevations. A study in *B. napus* revealed changes in cytosine methylation patterns under heat stress and variations in the degree of methylation in the plantlets of the two rapeseed cultivars, which were representative of genotypes that were heat-tolerant and heat-sensitive (Ghahramani *et al.*, 2019).

The methylation levels of the two genotypes differed during heat stress. Further evidence of increased methylation in the heat-sensitive genotype compared to the tolerant genotype was provided by the fact that the heat-tolerant genotype showed noticeably more DNA demethylation events than the sensitive genotype (Kim *et al.*, 2020). By altering cytosine methylation, the scientists found that exposure to heat affected several different gene sets. This implies that the vast majority of these genes play a role in heat stress adaptation and, ultimately, tolerance. Further elucidating the molecular mechanisms behind B. napus's adaptation to heat stress, this study also demonstrated that the heat-tolerant and heat-sensitive genotypes of the microorganism exhibit distinct DNA methylation modifications in response to heat stress (Zhang *et al.*, 2020). By raising genome methylation, the RNA-directed DNA methylation (RdDM) pathway upregulates the nuclear RNA polymerase NRPD1, NRPE1, and DRM2, which are significant epigenetic modulators in the response to heat, stress (Naydenov *et al.*, 2015) Table 2 contains more examples.

Tolerance to Cold Stress

Cold stress is believed to be a major environmental factor limiting crop production and agricultural expansion in mountainous terrain. The field of studying the epigenomes of plants exposed to cold is rapidly developing, according to Sanghera *et al.* (2011). Methylation-sensitive amplified fragment-length polymorphism markers detected changes in cytosine methylation in the alpine subnival plant Chorispora bungeana during exposure to freezing and chilling stress. Hu *et al.* (2011) discovered that cytosine methylation changed quickly during the freezing and chilling stages. Comparative methylome investigation of Populus simonii grown under osmotic, heat, salt, and cold shocks revealed condition-dependent variable cytosine methylation patterns and stress-specific differentially methylated regions (SDMRs) (Song *et al.*, 2013).

A recent study found that the chromatin remodeler facilitates the CBF-dependent cold tolerance of Arabidopsis. When pkl mutants are exposed to cold stress, they develop hypersensitivity (Yang *et al.*, 2019). Histone methylation is not the only histone change that has a substantial impact on the cold stress response. Histone acetylation is more prevalent in the sequence of many cold-responsive genes (Park *et al.*, 2018). Table 2 provides numerous examples.

Tolerance to heavy metal

At high quantities, heavy metals—metallic elements with large atomic weights and densities—can be harmful to plants and other living things. Cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), chromium (Cr), nickel (Ni), and other common heavy metals can have detrimental effects on plant health (Cong et al., 2024). These metals are frequently released into the environment by mining, urban runoff, industrial processes, and agricultural practices. Inhibition of Photosynthesis, Oxidative Stress, Disruption of Mineral Nutrient Uptake, Inhibition of Enzyme Activity, Alteration of Gene Expression, and Induction of Cell Death and Tissue Damage are some of the ways that heavy metals can impact plant health (Clemens and Ma, 2016). Epigenetic modifications in plants offer promising strategies for enhancing heavy metal resistance and improving plant survival and productivity in contaminated environments. For more examples see Table 2.

Overall, the table below provides an overview of the various applications of epigenetic modifications in crops particularly focusing on enhancing their tolerance to abiotic stresses (Table 2).

Table 2. Summary table application of epigenetic modification in crop response to different abiotic stresses.

Stress	Crop	Mechanism	Mechanisms and response	Reference
Heat	*Solanum tuberosum L.*	HM and demetylation	Heat stress response in potato	Mali and Zinta 2024
	Saccharina japonica	DNA methylation	Heat stress in *Saccharina japonica*	Liu *et al*., 2023
	Cucumber	lncRNAs, circRNAs, miRNAs	Analysis of co-expression and ceRNA networks under heat stress	He *et al*., 2020
	Zea mays	Histone acetylation	Nucleolar disorganization	Yue *et al*., 2021
Drought	*Oraya sativa*	DNA methylation	Drought stress tolerance	Kumar *et al*., 2023
	Tomato	RNA-directed DNA methylation	RNA-directed DNA methylation pathway involvement	Huang *et al*., 2016
	Arabidopsis thaliana	DNA demethylation	Improved water retention, increase transposon expression	Ashapkin *et al*., 2020
	Gossypium hirsutum	Histone acetylation	Improved drought tolerance by decreasing H3K9ac levels in the GhWRKY33 promoter	Zhang *et al*., 2020
	Triticulum aestivum	Histone methylation	Downregulated 5 HDA genes and upregulated TaHAC2 in drought-resistant BL207	Li *et al*., 2022
	Dendrobium hirsutum	Histone deacetylation	Under drought stress, the DoHDA10 and DoHDT4 genes are expressed in the roots, stems, and leaves.	Zhang *et al*., 2020
Cold stress	*Gossypium hirsutum*	Histone methylation	Cold response in upland cotton	Wang *et al*., 2024
	Rice	Histone deacetylation	Resistance to cold stress	Sun *et al*., 2024
	Arabidopsis thaliana	DNA methylation and Histone deacetylation	Silencing of detoxification pathway genes in ROXY19OE plants	Li *et al*., 2024
	Litopenaeus vannamei	DNA methylation	Cold stress tolerance in *Litopenaeus vannamei*	Zhu, *et al*., 2023
Salt stress	*Trichoderma*	Histone acetylation	Adaptation to salt stress	Li *et al*., 2022
	Soybean	DNA hypomethylation	Salt-stress priming	Yung *et al*., 2024
Heavy metal	*Orayaza sativa*	DNA methylation	Resistance to heavy metal mercury (Hg) stress	Cong *et al*., 2024
	Poplar	miRNA	Regulation of citric acid production and Pb uptake	Chen *et al*., 2022

Application of epigenetics in biotic stress

The expression of genes that give crop plants resistance to various biotic stressors involving bacterial, viral, and fungal pathogens is regulated in part by epigenetic mechanisms. Either host-specific defence response or basal defence is a part of the overall process of biotic stress tolerance. When various pathogens, such as nematodes or fungi, attack, basal defence activates pathogen-activated molecular patterns (PAMPs) (Zheng *et al*., 2021). Conversely, either canonical and non-canonical R genes or QTLs are activated in the host-specific response (Zheng *et al*., 2021). Plants that undergo epigenetic modifications in response to biotic stress may pass these modifications on to their offspring. Offspring of stressed plants may be more resilient to the same or related stressors due to the inheritance of epigenetic markers. This affects breeding plans that try to create crop varieties that can withstand stress. In reaction to biotic stress, gene expression is regulated by small RNA molecules, including microRNAs and small interfering RNAs. Epigenetic modifications can influence the production and activity of these small RNAs, thereby modulating the plant's defense responses.

Understanding how diseases affect their host plants has been made possible by epigenetic research. Certain infections can facilitate infection by changing the host's gene expression through epigenetic alterations. Comprehending these systems can facilitate the development of tactics to combat the pathogenicity of pathogens. Breeding programs that use epigenetic information can produce crops that are more resilient to biotic stress. Breeding can be accelerated to produce stress-tolerant types by identifying and choosing plants with advantageous epigenetic alterations (Zheng *et al.*, 2021).

Advances in CRISPR-based technologies have enabled targeted modifications of the epigenome. Therefore, epigenome editing can be employed to engineer specific epigenetic changes in plants to enhance their resistance to biotic stress (Zheng *et al.*, 2021).

Viruses

The modulation of viral virulence through Post transcriptional gene silencing (PTGS) was one of the earliest indications of the epigenetic regulation of plant tolerance to biotic stimuli (Ramirez *et al.*, 2018). When RNA viruses infect plants, they identify the double-strand RNA molecules and DCL2 and DCL4 break them down into siRNAs. Through PTGS, these siRNAs target viral RNA for degradation, limiting viral replication.

Plants have evolved a sophisticated RNA-based defense system to protect themselves from viral infections and control their own gene expression. Unlike animals, plants lack an adaptive immune system. Instead, they rely on mechanisms like post-transcriptional gene silencing (PTGS) and transcriptional gene silencing (TGS).

PTGS is activated when a plant is infected by a virus. The plant's defense system recognizes the viral RNA and cleaves it into small interfering RNAs (siRNAs). These siRNAs then target the viral RNA for degradation, preventing viral replication.

TGS is another important defense mechanism that involves the methylation of DNA and histones. This process can silence genes that are harmful to the plant, such as transposable elements. TGS can also be used to target viral DNA that has integrated into the plant genome. (Ramirez et al., 2018). Table 3 contains related examples.

Microbes

Numerous studies have been conducted on the function of DNA methylation in plant immunity (Ramirez *et al.*, 2018). Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), the initial line of active defence, depends on pattern-recognition receptors (PRRs) detecting PAMPs or microbe-associated molecular patterns (MAMPs). Basal immunity is the outcome of immune responses being triggered after PAMP awareness. Pathogens create unique effector chemicals that dampen PTI in order to overcome the plant defence. Disease resistance proteins may recognize these pathogen effectors as a counter-counter defence, which frequently results in a strong immune response known as effector-triggered immunity (ETI). Significant alterations in gene expression controlled by epigenetic processes are required for the activation of both PTI and ETI (Arruda *et al.*, 2022). Numerous related examples are shown in Table 3.

Pests

In the fields of plant biology and agriculture, a pest is any creature that damages or harms crops, lowering their production or quality (Yu and Peng, 2020). Pests' effects on plants include In addition to causing indirect plant damage through disease transmission, pests can cause direct plant damage by nibbling on leaves, stems, roots, or fruits, which can stunt plant development, diminish production, or even kill plants. For instance, diseases like mosaic, wilt, or blight can be spread by plant viruses, bacteria, or fungi by insect vectors (Barros *et al.*, 2021). Induced resistance, gene silencing, and epigenetic insecticides/fungicides are examples of how epigenetic modifications are used to control pests. Weeds compete with crops for resources like nutrients, water, and sunlight, resulting in lower crop yields and financial losses. Pests can cause stress responses in plants, causing physiological and biochemical changes that may affect plant growth, development, and productivity (Barros *et al.*, 2021). Table 3 contains related examples.

Parasitic Plants

In order to obtain nutrients and water for their own growth and reproduction, parasitic plants use specialized structures called haustoria to pierce the tissues of their hosts. Haustoria act as channels for the movement of signaling chemicals, proteins, DNA, and RNA in addition to nutrients and water (Gaut *et al.*, 2019). cDNA libraries were created from the stem sections of the host (Arabidopsis or tomato) that were devoid of parasites in order to detect the mobile transcriptomes of both hosts and parasites (Kim *et al.*, 2024). The majority of mobile transcripts came from hosts of Arabidopsis. Cuscuta included around 45% (9518) of the expressed Arabidopsis transcripts. On the other hand, Cuscuta only showed 1.6% (347) of the tomato transcripts that were expressed. In relation to transfer from parasite to host, just 0.8% (288) of the expressed Cuscuta mRNAs were found in tomato, compared to 24% (8655) in Arabidopsis. Implying that the host plant controls haustorial selection. It was not yet known why Arabidopsis and tomato have different haustorial selectivity. It most likely represents part of the tomato's active defence mechanisms against infection, namely the release of protective substances at the infection site (Kaiser *et al*., 2015).

It is known that certain sRNAs that travel between parasite and host plants have trans-specific functions (Alakonya *et al*., 2012). Deep sequencing was recently used to examine the expression of sRNA in *C. campestris* cultivated on A. thaliana (Shahid *et al*., 2018). In the host-parasitic interface, 76 *C. campestris* sRNA species, including 43 miRNAs, were markedly increased in comparison to the parasite stem. All of the information presented suggests that the dynamics of the "arms race" between parasite and host plants are influenced by epigenetic interactions. Table 3.

Overall, the table below provides an overview of the various applications of epigenetic modifications in crops particularly focusing on enhancing their tolerance to biotic stresses (Table 3).

Table 3. Summery table of application of epigenetic modification in crop response to different biotic stresses.

Stress	Crop	Mechanism	Response	Reference
Bacteria	Soybean	DNA methylation	Resistance to *Phytophthora sansomeana* root rot	Lee *et al*., 2024
	Cassava	Histone deacetylation	Resistance to cassava bacterial blight	Zhao *et al*., 2023
	Cassava	Histone acetylation	Reducing lesion stimulation disease	Zeng *et al*., 2023
	Rice	miRNA	miR393 suppresses auxin signaling, enhancing resistance to Xanthomonas	Bhar and Roy, 2023
Fungi	Cotton	DNA methylation	Resistant to *Verticillium dahlia*	Chen *et al*., 2023
	Maize	Histone phosphorylation	Resistance to Colletotrichum graminicola leaf infection	Agostini *et al*., 2023
	Rice	siRNA	siRNA derived from transposons regulates the Pigm locus, conferring resistance to Magnaporthe	Huang and Jin, 2022
	A.thaliana and tomato	Histone acetylation	Resistance to *Botrytis cinerea*	Crespo *et al*., 2018
Virus	*A. thaliana*	DNA and Histone methylation	Resistance to Turnip mosaic virus	Silvia *et al*., 2024
	Tomato	miRNA	Resistance to tomato yellow leaf curl virus	Romero *et al*., 2023
	Tomato	Histone Methylation	Resistant to curly stant virus	Mahomed, 2022
Nematode	Rice	RNAi	Resistance to root-knot nematodes	Meijer *et al*., 2023
	Tomato	DNA methylation	Resistance to Root-Knot Nematodes	Leonetti and Molinari, 2020
Herbicide resistance	Grass weed	Histone methylation and Demethylation	Resistance to broad spectrum herbicide	Sen *et al* ., 2020
	Zea mays	DNA methylation	Roundup® resistance	Tyczewska *et al*., 2021
Weed	*A. thaliana*	RNAi	Resistance to weed	Shahid *et al*., 2018

CHALLENGES AND FUTURE DIRECTIONS

The widespread application of epigenetic modifications in plant breeding faces several challenges. At a biological level, the identification of target genes, the durable maintenance of induced epialleles, and the potential for off-target effects pose significant challenges. Additionally, inappropriate legislation concerning new breeding techniques can impede research and the implementation of advanced epigenetic modifications, hindering the effective control of gene expression. While short-term heritability of DNA methylation patterns has been demonstrated, the long-term heritability and stability of induced methylation remain largely unexplored, with limitations in studying successive plant generations.

Despite these challenges, recent years have witnessed substantial progress in understanding the epigenetic regulation of gene expression in plants, especially in model crops like *Arabidopsis*. Researchers are actively working to identify genes involved in epigenetic changes and exploring the potential for manipulating epigenetic variations to improve crop traits. The development of genome editing tools, such as CRISPR/Cas9, holds promise for site-specific manipulation of DNA methylation, offering new avenues for crop improvement. However, comprehensive understanding of the complex interactions between crop genomes and their regulatory networks is essential for realizing the full potential of epigenetic research in crop development.

Epigenetic research may contribute to novel methods for crop development and improved environmental stress tolerance. Technologies that study both genotype and epigenotype enable the identification of key factors influencing phenotypes and responses to environmental cues. By unraveling the complexity of gene regulation through epigenetic mechanisms such as DNA methylation, short RNAs, and chromatin changes, researchers can target specific genes and transcriptional factors for crop enhancement. Although challenges persist, ongoing efforts are crucial for translating epigenetic knowledge into the development of climate-smart crops that can withstand various stresses, ultimately benefiting global food security.

CONCLUSION

Epigenetics, the study of heritable changes in gene function that do not involve alterations to the underlying DNA sequence, has emerged as a critical field of research in the context of crop improvement. Recent findings have underscored the significance of epigenetic modifications in enhancing the tolerance of crop plants to both biotic and abiotic stresses. These modifications, such as DNA methylation in specific regions and histone methylations, offer a promising avenue for plant breeders to develop crops with improved yield, quality, stress resistance, and nutritional/medicinal properties. Manipulating these epigenetic mechanisms presents an opportunity to create crops that are better adapted to changing environmental conditions, thereby contributing to global food security and promoting sustainable agriculture practices.

Moreover, the existence of an "epigenetic code" alongside the well-known genetic code is proposed, emphasizing the importance of understanding the interplay between genetic and epigenetic factors. DNA methylation and histone modifications, particularly H3K27me2/3 in response to stress, are identified as primary forces shaping the epigenetic landscape. Simultaneous research on genetic variants and epigenetic differences is essential for a comprehensive understanding of crop adaptation mechanisms. Furthermore, the application of RNAi technology in genetic manipulation offers a powerful tool for enhancing crop quality and addressing global challenges. This technology provides a means to ensure food safety by inhibiting the synthesis of naturally occurring toxic metabolites in edible plant parts. Ultimately, the integration of epigenetic insights and RNAi technology in crop improvement endeavors offers a complex approach to meeting the nutritional needs of the growing world population while ensuring sustainable and safe agricultural practices.

REFERENCES

1. Abdulraheem, M., Mukhtar, I., Yani, X., Abiodun, Y., Gregorio, C., Hao, Z. and Jiandong Hu. (2024). Mechanisms of Plant Epigenetic Regulation in Response to Plant Stress: Recent Discoveries and Implications. *Plants*. **2**: 163–171.
2. Abid, G., Mingeot, D. and Muhovski, Y. (2017). Analysis of DNA methylation patterns associated with drought stress response in faba bean (Vicia faba L.) using methylation-sensitive amplification polymorphism (MSAP). *Environmental and Experimental Botany*. **142**: 34–44.
3. Agostini, R.B., Ariel, F., Rius, S.P., Vargas, W.A. and Campos, V.A. (2023). Trichoderma root colonization in maize triggers epigenetic changes in genes related to the jasmonic and salicylic acid pathways that prime defenses against Colletotrichum graminicola leaf infection. *Journal of Experimental Botany*. **74**: 2016–2028.
4. Akil, A., Jerman, E., Laila F., Esraa, Y., Sujitha, S., Al-Kurbi A., and Khalid A. (2020). Reading between the (Genetic) Lines: How Epigenetics is Unlocking Novel Therapies for Type 1 Diabetes. *Cells*. **9**: 2403–2432.
5. Alakonya, A., Kumar, R., Koenig, D., Kimura, S., Townsley, B., Runo, S., Garces, H.M., Kang, J., Yanez, A., David-Schwartz, R. and Machuka, J. (2012). Interspecific RNA interference of SHOOT MERISTEMLESS-like disrupts Cuscuta pentagona plant parasitism. *The Plant Cell*. **7**: 3153–3166.
6. Arruda, N.L., Bryan, A.F. and Dowen, J.M. (2022). PDS5A and PDS5B differentially affect gene expression without altering cohesin

localization across the genome. *Epigenetics and Chromatin*. **15**: 30–44.

7. Ashapkin, V.V., Kutueva, L.I., Aleksandrushkina, N.I. and Vanyushin, B.F. (2020). Epigenetic Mechanisms of Plant Adaptation to Biotic and Abiotic Stresses. *International journal of molecular sciences*. **21**: 7457–7468

8. Avramidou, E., Kapazoglou, A., Aravanopoulos, F., Xanthopoulou, A., Ganopoulos, I., Tsaballa, A., Madesis, P., Doulis, G. and Tsaftaris, A. (2015). Global DNA methylation changes in Cucurbitaceae inter-species grafting. *Crop Breeding and Applied Biotechnology*. **15**: 112–116.

9. Barros, S., DaRocha, M. and Batista, A. (2021). Epigenetic control as a potential mechanism for plant tolerance to abiotic stress. *International Journal of Molecular Science*. **13**: 7026–7042.

10. Bhadouriya, S.L., Mehrotra, S., Basantani, M.K., Loake, G.J. and Mehrotra, R. (2020). Role of Chromatin Architecture in Plant Stress Responses: An Update. *Frontiers in Plant Science*. **11**: 603–617.

11. Bhar, A. and Roy, A. (2023). Emphasizing the role of long non-coding RNAs (lncRNA), circular RNA (circRNA), and micropeptides (miPs) in plant biotic stress tolerance. *Plants*. **12**: 3951–3966.

12. Brukhin, V. and Albertini, E. (2021). Epigenetic Modifications in Plant Development and Reproduction. *Epigenomes*. **5**: 25–37.

13. Brzezinka, K., Altmann, S., Czesnick, H., Nicolas, P., Gorka, M., Benke, E., Kabelitz, T., Jähne, F., Graf, A., Kappel, C. and Bäurle, I. (2016). Arabidopsis FORGETTER1 mediates stress-induced chromatin memory through nucleosome remodeling. *elife*, **5**: 17061170623.

14. Castro, P. H., Verde, N., Lourenço, T., Magalhães, A. P., Tavares, R. M. and Bejarano, E. R. (2018). SUMO proteases ULP1c and ULP1d are required for development and osmotic stress responses in Arabidopsis thaliana. *Plant Molecular Biology*. **97**: 29–45.

15. Chachar, S., Chachar, M., Riaz, A., Shaikh, A., Li, X., Guan, C. and Zhang, P. (2022). Epigenetic modification for horticultural plant improvement comes of age. *Scientia Horticulturae*. **292**: 110633–110645.

16. Chen, C., Wang, M., Zhu, J., Tang, Y., Zhang, H., Zhao, Q., Jing, M., Chen, Y., Xu, X., Jiang, J. and Shen, Z. (2022). Long-term effect of epigenetic modification in plant–microbe interactions: modification of DNA methylation induced by plant growth-promoting bacteria mediates promotion process. *Microbiome*. **10**: 36–48.

17. Chen, L., Ge, B., Casale, F.., Vasquez, L., Kwan, T., Garrido-Martín, D., Watt, S., Yan, Y., Kundu, K., Ecker, S. and Datta, A. (2016). Genetic drivers of epigenetic and transcriptional variation in human immune cells. *Cell*. **5**: 1398–1414.

18. Chen, M., Lv, S. and Meng, Y., 2010. Epigenetic performers in plants. Development, *growth and differentiation*. **6**: 555–566.

19. Chen, X., He, L., Li, Y., Zhang, Y. and Chen, W. (2022). Long non-coding RNAs (lncRNAs) response to lead (Pb 2+) toxicity in *Populus tomentosa*. *Environmental and Experimental Botany*. **194**: 104682–104701.

20. Chen, Y., Hong, T., Wang, S., Mo, J., Tian, T. and Zhou, X. (2017). Epigenetic modification of nucleic acids: from basic studies to medical applications. *Chemical Society Reviews*. **10**: 2844–2872.

21. Chen, Y., Zhu, C., Zhao, J., Liu, T., Gao, F., Zhang, Y. and Duan, C. (2023). DNA methylation-dependent epigenetic regulation of Verticillium dahliae virulence in plants. *Abiotech*. **4**: 185–201.

22. Cheng, Y.H., Peng, X.Y., Yu, Y.C., Sun, Z.Y. and Han, L. (2019). The Effects of DNA Methylation Inhibition on Flower Development in the Dioecious Plant Salix Viminalis. *Forests*. **10**: 173–184.

23. Christman, J.K. (2002). 5-Azacytidine and 5-aza-2′-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene*. **35**: 5483–5495.

24. Clemens, S. and Ma, J. F. (2016). Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annual Review of Plant Biology*. **67**: 489–512.

25. Cong, W., Li, N., Miao, Y., Huang, Y., Zhao, W., Kang, Y., Zhang, B., Wang, J., Zhang, J., Lv, Y. and Li, J. (2024). DNA hypomethylation-associated transcriptional rewiring enables resistance to heavy metal mercury (Hg) stress in rice. *Journal of Hazardous Materials*. **41**: 132–139.

26. Crespo, S. Ó., Sánchez, G.L., López, G. M., Fernández, C.E., Schalschi, L.L., García, R. I., Rausell, C., Real, M.D. and González,B. C. (2020).The histone marks signature in exonic and intronic regions is relevant in early response of tomato genes to Botrytis cinerea and in miRNA regulation. *Plants.* **9**: 300–313.
27. Crowl, S., Coleman, M.B., Chaphiv, A. and Naegle, K.M., 2024. A systematic analysis of the effects of splicing on the diversity of post-translational modifications in protein isoforms. bioRxiv.
28. Dadami, E., Dalakouras, A., Zwiebel, M., Krczal, G. and Wassenegger, M. (2014). An endogene-resembling transgene is resistant to DNA methylation and systemic silencing. *RNA biology*. **11**: 934–941.
29. Dalakouras, A. and Ganopoulos, I. (2021). Induction of promoter DNA methylation upon high-pressure spraying of double-stranded RNA in plants. *Agronomy*. **11**: 789–794.
30. Dalakouras, A. and Vlachostergios, D. (2021). Epigenetic approaches to crop breeding: current status and perspectives. *Journal of Expermental Botany*. **72**: 5356–5371.
31. Dong, Q., Zhai, Y., Zhang, T., Hu, G., Qin, Y., Huang, Z. and Wang, Y. (2020). SWI3B and HDA6 interact and are required for transposon silencing in Arabidopsis. *The Plant Journal*. **102**: 471–484.
32. Duarte-Aké, F., Us-Camas, R. and De-la-Peña, C. (2023). Epigenetic Regulation in Heterosis and Environmental Stress: The Challenge of Producing Hybrid Epigenomes to Face Climate Change. *Epigenomes*.**7**: 14–25.
33. Ferreira, L.J., Azevedo, V., Maroco, J., Oliveira, M. and Santos, A. P. (2015). Salt Tolerant and Sensitive Rice Varieties Display Differential Methylome Flexibility under Salt Stress. *PLoS One*. **10**: 5–21.
34. Gale, C.R., Marioni, R.E., Harris, S.E., Starr, J.M. and Deary, I.J. (2018). DNA methylation and the epigenetic clock in relation to physical frailty in older people: the Lothian Birth Cohort 1936. *Clinical epigenetics*. **10**: 1–8.
35. Gallego-Bartolomé, J. (2020). DNA methylation in plants: mechanisms and tools for targeted manipulation. *New Phytologist*. **227**: 38–44.
36. Gaut, B.S., Miller, A.J. and Seymour, D.K. (2019). Living with two genomes: Grafting and its implications for plant. *Annual Review of Genetics*. **12**: 234–243.
37. Gehring, M. (2019). Epigenetic dynamics during flowering plant reproduction: evidence for reprogramming? *New Phytologist*. **224**: 91–96.
38. Geng, F., Zhang, Y., Lee, R. and Wang, Y. (2018). Biotinylated histone H2A and H2B in mammalian cells as a newly identified epigenetic mark. Protein. *Cell*. **9**: 675–682.
39. Ghahramani, A., Howden, S., Del Prado, A., Thomas, D., Moore, A., Ji, B. and Ates, S. (2019). Climate Change Impact, Adaptation, and Mitigation in Temperate Grazing Systems: A Review*: Sustainability*. **11**: 7224–7231.
40. Gupta, C. and Salgotra, R. (2022). Epigenetics and its role in effecting agronomical traits. *Frontiers in Plant Science*. **13**: 925–988.
41. Han, Q., Bartels, A., Cheng, X., Meyer, A., An, Y.Q., Hsieh, T.F. and Xiao, W. (2019). Epigenetics regulates reproductive development in plants. *Plants*. **8**: 564–571.
42. Hang, H., Zhang, X. and Xiao, J. (2023). Epigenetic Regulation of Nitrogen Signaling and Adaptation in Plants. *Plants.* **12**: 2725–2733.
43. He, M.Y., Ren, T.X.,Jin, Z.D., Deng, L., Liu, H.J., Cheng, Y.Y., Li, Z.Y., Liu, X.X.,Yang, Y. and Chang, H. (2023). Precise analysis of potassium isotopic composition in plant materials by multi-collector inductively coupled plasma mass spectrometry. *Atomic Spectroscopy*. **209**: 10–24.
44. He, X., Guo, S., Wang, Y., Wang, L., Shu, S. and Sun, J. (2020). Systematic identification and analysis of heat-stress-responsive lncRNAs, circRNAs and miRNAs with associated co-expression and ceRNA networks in cucumber (*Cucumis sativus* L.). *Physiologia plantarum*. **168**: 736–754.
45. Hirakawa, T., Tanno, S. and Ohara, K. (2024). Exogenous treatment with N-acetylglutamic acid confers tolerance to heat stress in plants. Plant Biotechnology. **41**: 71–76.
46. Holliday, R. and Pugh, J.E. (1996). DNA modification mechanisms and gene activity during development. *Cold Spring Harbor Monograph Series*, **32**: 639–645.
47. Hou, H., Zhao, L., Zheng, X., Gautam, M., Yue, M., Hou, J., Chen, Z., Wang, P. and Li, L. (2019). Dynamic changes in histone modification are associated with upregulation of Hsf and rRNA

genes during heat stress in maize seedlings. *Protoplasma*. **256**: 1245–1256.

48. Hou, Y., Wang, L., Wang, L., Liu, L., Li, L., Sun, L. and Xie, Q. (2020). SUMOylation of MYB30 enhances salt tolerance by elevating alternative respiration via transcriptionally upregulating AOX1a in Arabidopsis. *The Plant Journal.* **18**: 1731–1743.

49. Hu, Y., Zhang, L., Zhao, L., Li, J., He, S., Zhou, K., Yang, F., Huang, M., Jiang, L. and Li, L. Trichostatin, A. (2011). Selectively Suppresses the Cold-Induced Transcription of the ZmDREB1 Gene in Maize. *PLoS One*. **6**: 197–208.

50. Huang, C. and Jin, H. (2022). Coordinated epigenetic regulation in plants: a potent managerial tool to conquer biotic stress. *Frontiers in Plant Science*. **12**: 79–89.

51. Huang, W., Xian, Z., Hu, G. and Li, Z. (2016). SlAGO4A, a core factor of RNA-directed DNA methylation (RdDM) pathway, plays an important role under salt and drought stress in tomato. *Molecular Breeding*. **36**: 1–13.

52. Hung, Y.H. and Slotkin, R.K. (2021). The initiation of RNA interference (RNAi) in plants. *Current Opinion in Plant Biology*. **61**: 102–115.

53. Huo, Y., Ma, Z., Chen, Z., Zhuang, L., Yang, Y. and Wang, X. (2018). Biotin repressor: A novel regulator for heterologous expression in *Alicyclobacillus acidoterrestris*. *Journals of Biotechnology*. **284**: 1–6.

54. Jogam, P., Sandhya, D., Alok, A., Peddaboina, V., Allini, V. and Zhang, B. (2023). A review on CRISPR/Cas-based epigenetic regulation in plants. *International Journal of Biological Macromolecules*. **219**: 1261–1271.

55. Kaiser, B., Vogg, G., Fürst, U.B. and Albert, M. (2015). Parasitic plants of the genus Cuscuta and their interaction with susceptible and resistant host plants. *Frontiers in plant science*. **6**: 45–56

56. Kajal, S., Aalok, S., Lorenna, L., Karansher, S., Parichita, P. and Sourav, R. (2021). A comprehensive review on epigenetic mechanisms and application of epigenetic modifications for crop improvement. *Environmental and Experimental Botany*. **188**: 104–179.

57. Kakoulidou, I., Avramidou, E., Baránek, M., Brunel-Muguet, S., Farrona, S., Johannes, F., Kaiserli, E., Lieberman-Lazarovich, M., Martinelli, F., Mladenov, V., Testillano, P., Vassileva, V., Maury, S. (2021) Epigenetics for Crop Improvement in Times of Global Change. *Biology*. **10**: 766–787.

58. Kikuchi, M., Morita, S., Wakamori, M., Sato, S., Uchikubo-Kamo, T., Suzuki, T., Dohmae, N., Shirouzu, M. and Umehara, T. (2023). Epigenetic mechanisms to propagate histone acetylation by p300/CBP. *Nature Communications*. **14**: 4103–4131.

59. Kim, J., To, T. K., Ishida, J., Henz, S. R., Yamagishi, K., & Kim, C. Y. (2006). A novel class of chromatin remodeling factor essential for epigenetic regulation of stress-responsive genes in Arabidopsis. *Genes and Development*. **20**: 1497–1509).

60. Kim, J.H. (2019). Chromatin Remodeling and Epigenetic Regulation in Plant DNA Damage Repair. *International Journal of Molecular Sciences*. **20**: 40–93.

61. Kim, J. H. (2020). Multifaceted Chromatin Structure and Transcription Changes in Plant Stress Response. *International Journal of Molecular Sciences*. **22**: 2013–2026.

62. Kim, J. M., Kim, S. R., Kim, J. H., Kim, H. Y. and Park, C. S. (2020). DNA methylation and histone modifications in response to abiotic stress in plants. *International Journal of Molecular Sciences*. **21**: 5125–5134.

63. Kim, J.H., Kim, M.S. and Seo, Y.W. (2024). Overexpression of a TaATL1 encoding RING-type E3 ligase negatively regulates cell division and flowering time in Arabidopsis thaliana. *Plant Science*. **340**: 111966–111977.

64. Kumar, J., Rai, K., Pirseyedi, S., Elias, M., Xu, S., Dill-Macky, R. and Kianian, S. F. (2017). Epigenetic regulation of gene expression improves Fusarium head blight resistance in durum wheat. *Scientific reports*. **10**: 176–200.

65. Kumar, M. and Rani, K. (2023). Epigenomics in stress tolerance of plants under the climate change. *Molecular Biology Reports*. **50**: 1–16.

66. Kumari, P., Khan, S., Wani, I. A., Gupta, R., Verma, S., Alam, P. and Alaklabi, A. (2022). Unravelling the role of epigenetic modifications in development and reproduction of angiosperms: A critical appraisal. *Frontiers in genetics*.**13**: 819–941.

67. Lanata, C.M., Chung, S.A., Criswell, L. A. (2018). DNA methylation 101: what is important to know about DNA methylation and its role in SLE risk and disease heterogeneity. *Lupus science and medicine*. **25**: 1–15.
68. Lang, J., McKie, J., Smith, H., McLaughlin, A., Gillberg, C., Shiels, P. and Minnis, H. (2017). Adverse childhood experiences, epigenetics and telomere length variation in childhood and beyond: a systematic review of the literature. *European child and adolescent psychiatry*. **29**: 1329–1338.
69. Lee, G., DiBiase, C.N., Liu, B., Li, T., McCoy, A.G., Chilvers, M.I., Sun, L., Wang, D., Lin, F. and Zhao, M. (2024). Transcriptomic and epigenetic responses shed light on soybean resistance to Phytophthora sansomeana. *The Plant Genome*. **12**: 2024–2060.
70. Lee, S., Choi, J., Park, J., Hong, C.P., Choi, D., Han, S., Choi, K., Roh, T.Y., Hwang, D. and Hwang, I. D. (2023). Mediated gene body DNA methylation is associated with inducible activation of defense-related genes in Arabidopsis. *Genome Biology*. **24**: 106–127.
71. Leonetti, P. and Molinari, S. (2020). Epigenetic and Metabolic Mechanisms in Plant Resistance to Root-Knot Nematodes. International *Journal of Molecular Science*. **21**: 59–77.
72. Li, A., Hu, B. and Chu, C. (2021). Epigenetic regulation of nitrogen and phosphorus responses in plants. *Journal of plant physiology*. **258**: 153363–15338.
73. Li, H. and Yang, B. (2013). Friend or foe: the role of microRNA in chemotherapy resistance. *Acta pharmacologica sinica*. **34**: 870–879.
74. Li, H., Liu, H., Pei, X., Chen, H., Li, X., Wang, J. and Wang, C. (2022). Comparative Genome-Wide Analysis and Expression Profiling of Histone Acetyltransferases and Histone Deacetylases Involved in the Response to Drought in Wheat. *Journal of Plant Growth Regulation*. **41**: 1065–1078.
75. Li, N., Yu, P., Zeng, Y., Chen, J., Yang, W., Qin, G., Du, S., Han, X. and Huang, L.J. (2024). Characterization of epigenetic modifications in a plant-specific glutaredoxin-mediated repression of stress-responsive gene expression. Environmental and Experimental Botany. **217**: 105582–105597.
76. Li, X., Qian, W., Zhao, Y., Wang, C., Shen, J., Zhang, L. and Gong, Z. (2016). Antisilencing role of the RNA-directed DNA methylation pathway and a histone acetyltransferase in Arabidopsis. *Proceedings of the National Academy of Sciences*. **113**: 11455–11460.
77. Li, Y., Li, Z., Dong, L., Tang, M., Zhang, P., Zhang, C., Cao, Z., Zhu, Q., Chen, Y., Wang, H. and Wang, T. (2018). Histone H1 acetylation at lysine 85 regulates chromatin condensation and genome stability upon DNA damage. *Nucleic acids research*. **46**: 7716–7730.
78. Li, Z., Zhang, H., Cai, C., Lin, Z., Zhen, Z., Chu, J. and Guo, K. (2022). Histone acetyltransferase GCN5-mediated lysine acetylation modulates salt stress aadaption of Trichoderma. *Applied Microbiology and Biotechnology*. **8**: 3033–3049.
79. Lin, X., Lu, Y., Zhang, C., Cui, Q., Tang, Y.D., Ji, X. and Cui, C. (2024). LncRNA Disease v3. 0: an updated database of long non-coding RNA-associated diseases. *Nucleic Acids Research*. **52**: 1365–1369.
80. Liu, F., Zhang, P., Liang, Z., Yuan, Y., Liu, Y. and Wu, Y. (2023). The global dynamic of DNA methylation in response to heat stress revealed epigenetic mechanism of heat acclimation in Saccharina japonica. *Journal of Phycology*. **59**: 249–263.
81. Liu, Y., Priyadarshani, N., Chi, M., Yan, M., Mohammadi, M., Zhang, M., Zhou, Q., Wang, L., Luo, T., Wai, M. and Wang, X. (2023). Epigenetic modification mechanisms of chloroplasts mutants in pineapple leaves during somatic regeneration. *Horticultural Plant Journal*. **9**: 509–522.
82. Liu, Y., Wang, J., Liu, B. and Xu, Z. (2022). Dynamic regulation of DNA methylation and histone modifications in response to abiotic stresses in plants. *Journal of Integrative Plant Biology*. **64**: 2252–2274.
83. Lucibelli, F., Maria, C., Valoroso, A. and Serena A. (2022). "Plant DNA Methylation: An Epigenetic Mark in Development, Environmental Interactions, and Evolution. *International Journal of Molecular Sciences*. **15**: 8299–8313.
84. Luo, M., Sarnowski, T.J., Libault, M., Ríos, G., Charron, J.B., Mantri, N. and Zhang, S. (2021). New insights into mechanisms of epigenetic modifiers in plant growth and development, volume II. *Frontiers in plant science*. **14**: 121–141.
85. Lv, Y., Li, J., Wang, Z., Liu, Y., Jiang, Y., Li, Y., Lv, Z., Huang, X., Peng, X., Cao, Y. and Yang, H.,

2024. Polycomb proteins RING1A/B promote H2A monoubiquitination to regulate female gametophyte development in Arabidopsis. *Journal of Experimental Botany*. **75**: 4822–4836.

86. Mahomed, N.B. (2022). Investigation of histone methylation of tomato curly stunt virus (ToCSV) in resistant and susceptible tomato lines (Doctoral dissertation, University of Johannesburg). *Frontiers in plant science*. **14**: 1135442–1135467.

87. Mali, S. and Zinta, G. (2024). Genome-wide identification and expression analysis reveal the role of histone methyltransferase and demethylase genes in heat stress response in potato (*Solanum tuberosum* L.). *General Subjects*. **1868**: 130507–130519.

88. Martienssen, R. A. and Colot, V. (2001). Transposable elements: a genomic perspective. Science. **291**: 1225–1226.

89. Martínez, M., Aparicio, F., López-Gresa, M., Bellés, J., Sánchez, J. and Pallás, V. (2017). Arabidopsis m6A demethylase activity modulates viral infection of a plant virus and the m6A abundance in its genomic RNAs. *Proceedings of the National Academy of Sciences*. **114**: 10755–10760.

90. Martínez-Pérez, M., Gómez-Mena, C., Alvarado-Marchena, L., Nadi, R., Micol, J.L., Pallas, V. and Aparicio, F. (2021). The m6A RNA demethylase ALKBH9B plays a critical role for vascular movement of alfalfa mosaic virus in Arabidopsis. *Frontiers in Microbiology*. **12**: 7455765–7458574.

91. Mattick, J.S., Amaral, P.P., Carninci, P., Carpenter, S., Chang, H.Y., Chen, L.L., Chen, R., Dean, C., Dinger, M.E., Fitzgerald, K.A. and Gingeras, T.R. (2023). Long non-coding RNAs: definitions, functions, challenges and recommendations. *Nature reviews Molecular cell biology*. **24**: 430–447.

92. Mazur, R., Gieczewska, K., Kowalewska, Ł., Kuta, A., Proboszcz, M., Gruszecki, W.I., Mostowska, A. and Garstka, M. (2020). Specific composition of lipid phases allows retaining an optimal thylakoid membrane fluidity in plant response to low-temperature treatment. *Frontiers in Plant Science*. **11**: 723–741.

93. Meijer, A., Atighi, M., Demeestere, K., De Meyer, T., Vandepoele, K. and Kyndt, T. (2023). Dicer-like 3a mediates intergenerational resistance against root-knot nematodes in rice via hormone responses. *Plant. Physiology*. **193**: 2071–2085.

94. Mercé, C., Bayer, P.E., Tay Fernandez, C., Batley, J. and Edwards, D. (2020). Induced methylation in plants as a crop improvement tool: progress and perspectives. *Agronomy*. **10**: 1484–1501.

95. Migliorini, F., Vecchio, G., Giorgino, R., Eschweiler, J., Hildebrand, F. and Maffulli, N. (2023). Micro RNA in meniscal ailments: current concepts. *British Medical Bulletin*. **145**: 141–6150.

96. Minorsky, V. (2014) "On the Inside." Plant Physiology **165**: 477–494

97. Miranda, T.B., Cortez, C. C., Yoo, C.B., Liang, G., Abe, M., Kelly, T.K., Marquez, V.E. and Jones, P. A. (2009). DZNep is a global histone methylation inhibitor that reactivates developmental genes not silenced by DNA methylation. *Molecular cancer therapeutics*. **8**: 1579–1588.

98. Mittler, R. (2006). Abiotic stress signal transduction. *Annual Review of Plant Biology*. **57**: 209–236.

99. Nowicka, A., Tokarz, B. and Zwyrtkova, J. (2020). Comparative analysis of epigenetic inhibitors reveals different degrees of interference with transcriptional gene silencing and induction of DNA damage. *The Plant Journal*. **102**: 68–84.

100. Ong-Abdullah, M., Ordway, J. and Jiang, N. (2015). Loss of Karma transposon methylation underlies the mantled soma clonal variant of oil palm. *Nature*. **525**: 533–537.

101. Onufriev, A.V. and Schiessel, H. (2019). The nucleosome: from structure to function through physics. *Current opinion in structural biology*. **56**: 119–156.

102. Pandey, G., Sharma, N., Pankaj Sahu, P. and Prasad, M. (2016). Chromatin-based epigenetic regulation of plant abiotic stress response. *Current Genomics*. **6**: 490–498.

103. Park, J., Lim, C., Shen, M., Park, H., Cha, J., Iniesto, E., Rubio, V., Mengiste, T., Zhu, J. and Bressan, R. (2018). Epigenetic switch from repressive to permissive chromatin in response to cold stress. *Proceedings of the National Academy of Sciences*. **115**: 400–5409.

104. Pecinka, A., Chevalier, C., Colas, I., Kalantidis, K., Varotto, S., Krugman, T., Michailidis, C., Vallés, M., Muñoz, A. and Pradillo, M. (2020). Chromatin dynamics during interphase and cell division: Similarities and differences

between model and crop plants. *Journal of Experimental Botany*. **71**: 5205–5222.

105. Preez, P.H., Breeds, K., Burger, N.F.V., Swiegers, H.W., Truter, J.C. and Botha, A.M., 2020. DNA Methylation and demethylation are regulated by functional DNA methyltransferases and DnTET enzymes in Diuraphis noxia. *Frontiers in Genetics*. 11: 452–467.

106. Qi, Q., Hu, B., Jiang, W., Wang, Y., Yan, J., Ma, F., Guan, Q. and Xu, J. (2023). Advances in plant epigenome editing research and its application in plants. *International Journal of Molecular Sciences*. **24**: 34–42.

107. Rahikainen, M., Pascual, J., Alegre, S., Durian, G., Kangasjärvi, S., Tikkanen, M. and Kangasjärvi, J. (2019). Biotin influences acetyl-CoA carboxylase 2 domain biotinylation and activity in Arabidopsis. *Plant Direct*. **3**: 15–36

108. Ramirez, J.S., Abulfaraj, A. A.,Rayapuram, N., Benhamed, M. and Hirt, H. (2018). Plant immunity: From signaling to epigenetic control of defense. *Trends in plant science*. **23**: 833–844.

109. Romero, B., Petek, M. and Jiao, C. (2023). Transcriptional and epigenetic changes during tomato yellow leaf curl virus infection in tomato. *BMC Plant Biology*. **23**: 651–672.

110. Saktiyono, Suwarto., Suprayogi and Noor. (2022). Drought Stress: Responses and Mechanism in Plants. *Reviews in Agricultural Science*. **10**: 168–185.

111. Sanghera, G., Wani, S., Hussain, W. and Singh, N. (2011). Engineering Cold Stress Tolerance in Crop Plants. *Current genomics*. **12**: 30–43.

112. Sen, M., Hamouzova, K., Košnarová, P., Roy, A. and Soukup, J. (2011). Herbicide resistance in grass weeds: Epigenetic regulation matters too. *Frontier Plant Science*. **13**:1040958.

113. Sen, M.K., Hamouzová, K., Košnarová, P., Roy, A. and Soukup, J. (2022). Herbicide resistance in grass weeds: Epigenetic regulation matters too. *Frontiers in Plant Science*. **13**: 1040958–100967.

114. Shahid, S., Kim, G., Johnson, N., Wafula, E., Wang, F., Coruh, C., Bernal, G. and Phifer, T. (2018). MicroRNAs from the parasitic plant Cuscuta campestris target host messenger RNAs. *Nature*. **86**: 82–85.

115. Silvia, A., María, J., Olmo,U., Régis, L., Corrêa, S. and Elena, F. (2024). Phenotypic and genomic changes during Turnip mosaic virus adaptation to Arabidopsis thaliana mutants lacking epigenetic regulatory factors. *Evolution*. **78**: 69–85.

116. Song, X., Zhang, Y., Zhong, Q., Zhan, K., Bi, J., Tang, J., Xie, J. and Li, B. (2020). Identification and functional characterization of methyl-CpG binding domain protein from Tribolium castaneum. *Genomics*. **112**: 2223–2232.

117. Song, Y., Ito, S. and Imaizumi, T. (2013) Flowering time regulation: Photoperiod- and temperature-sensing in leaves. *Trends Plant Science*. **18**: 575–583.

118. Song, Z.T., Liu, J.X. and Han, J.J., 2021. Chromatin remodeling factors regulate environmental stress responses in plants. *Journal of Integrative Plant Biology*. **63**:438–450.

119. Spillane, C. and McKeown, P.C. (2020). Plant epigenetics and epigenomics. *Springe*r. **7**: 2093–3001.

120. Suleiman, A., Al-Chalabi, R. and Shaban, A. (2024). Integrative role of small non-coding RNAs in viral immune response: a systematic review. *Molecular Biology Reports*. **51**: 1–12.

121. Sun, C., Ali, K., Yan, K., Fiaz, S., Dormatey, R., Bi,Z. and Bai, J. (2021). Exploration of Epigenetics for Improvement of Drought and Other Stress Resistance in Crops: *A review. Plants*. **10**: 1226–1234.

122. Sun, Y., Xie, Z., Jin, L., Qin, T., Zhan, C. and Huang, J. (2024). Histone deacetylase OsHDA716 represses rice chilling tolerance by deacetylating OsbZIP46 to reduce its transactivation function and protein stability. *The Plant Cell*. **51**: 1–12.

123. Surdonja, K., Eggert, K., Hajirezaei, M., Harshavardhan, V., Seiler, C., Von Wirén, N., Sreenivasulu, N. and Kuhlmann, M. (2017). Increase of DNA Methylation at the HvCKX2.1 Promoter by Terminal Drought Stress in Barley. *Epigenomes*. **1**: 9–17.

124. Thieme, M., Lanciano, S., Balzergue, S., Daccord, N., Mirouze, M. and Bucher, E. (2017). Inhibition of RNA polymerase II allows controlled mobilization of retrotransposons for plant breeding. *Genome Biology*. **18**: 134–147.

125. Tonosaki, K., Fujimoto, R., Dennis, E.S., Raboy, V. and Osabe, K. (2022). Will epigenetics be a key player in crop breeding? *Fronter Plant Science*. **13**: .95–113.

126. Tyczewska, A., Gracz-Bernaciak, J., Szymkowiak, J. and Twardowski, T. (2021). Herbicide stress-induced DNA methylation changes in two Zea mays inbred lines differing in Roundup® resistance. Journal of Applied Genetics, **62**: .235–248.

127. Ueda, M. and Seki, M. (2020). Histone modifications form epigenetic regulatory networks to regulate abiotic stress response. *Plant Physiology*. **182**: 15–26.

128. Uthup, T.K., Karumamkandathil, R., Ravindran, M. and Saha T. (2018). Heterografting induced DNA methylation polymorphisms in Hevea brasiliensis. *Planta.* **248**: 579–589.

129. Varotto, S., Tani, E., Abraham, E., Krugman, T., Kapazoglou, A., Melzer, R., Radanović, A. and Miladinović, D. (2020). Epigenetics: possible applications in climate-smart crop breeding. *Journal of Botany*. **71**: 5223–5236.

130. Ventouris, Y. E., Tani, E., Avramidou, E.V. and Abraham, E.M., Chorianopoulou, S.N., Vlachostergios, D.N., Papadopoulos, G. and Kapazoglou, A. (2020). Recurrent Water Deficit and Epigenetic Memory in Medicago sativa L. Varieties. *Appl. Sci*. **10**: 3110–3124.

131. Wang, X., Guo, Y., Wang, Y., Peng, Y., Zhang, H. and Zheng, J. (2024). ZmHDT103 Negatively Regulates Drought Stress Tolerance in Maize Seedlings. *Agronomy*. **14**: 134–147.

132. Wang, Y., Zhang, T., Wang, R., Zhao, Y., Wang, Y., Xu, Y. and Li, Z. (2020). Arabidopsis scaffold protein RACK1A interacts with diverse environmental stress responses. *Molecular Plant*. **13**: 150–164.

133. Wang, Z., Cao, H., Chen, F., Liu, Y. and Mi, G. (2019). Histone modification dynamics during early seed development in Arabidopsis. *Planta.* **249**: 725–739.

134. Wu, C.J., Yuan, D.Y., Liu, Z.Z., Xu, X., Wei, L., Cai, X.W., Su, Y.N., Li, L., Chen, S. and He, X.J. (2023). Conserved and plant-specific histone acetyltransferase complexes cooperate to regulate gene transcription and plant development. *Nature Plants*. **9**: 442–459.

135. Xiao, X., Liu, X. and Jiao, B. (2020). Epigenetics: Recent advances and its role in the treatment of Alzheimer's disease. *Frontiers in Neurology.* **11**: 53–76.

136. Yaacob, J.S., Loh, H.S. and Mat Taha, R. (2013). Protein profiling and histone deacetylation activities in somaclonal variants of oil palm (Elaeis guineensis Jacq.). *The Scientific World Journal*. **6**: 136–155.

1347. Yamaguchi, N. (2022). Epigenetics in Plant Development. *Frontiers in Plant Science*. **13**: 864–945.

138. Yang, R., Hong, Y., Ren, Z., Tang, K., Zhang, H., Zhu, J. and Zhao, C. (2019). A Role for PICKLE in the Regulation of Cold and Salt Stress Tolerance in Arabidopsis. *Frontiers in Plant Sci*ence. **10**: 900–151.

139. Yu, S., Dang, D., Yin, Y., Dai, Y., Zhang, X., Sun, Y., Dong, X. and Ruan, Y. (2023). Expression and biogenesis analysis of 21-nt and 24-nt phased small interfering RNAs in maize tissues. *Planta*. **187**: 226–232.

140. Yu, X. and Peng, Y. (2020). A deeper understanding of epigenetics in crop pest control. *Frontiers in Plant Science*. **11**: 8–19.

141. Yue, M., Gautam, M., Chen, Z., Hou, J., Zheng, X., Hou, H. and Li, L. (2021). Histone acetylation of 45S rDNA correlates with disrupted nucleolar organization during heat stress response in *Zea mays* L. *Physiologia Plantarum*. **172**: 2079–2089.

142. Yung, W.S., Wang, Q., Chan, L.Y., Wang, Z., Huang, M., Li, M.W., Wong, F.L. and Lam, H.M. (2024). DNA Hypomethylation Is One of the Epigenetic Mechanisms Involved in Salt-Stress Priming in Soybean Seedlings. *Plant Cell and Environment*.

143. Zeng, H., Xu, H., Tan, M., Zhang, B. and Shi, H. (2023). LESION SIMULATING DISEASE 3 regulates disease resistance via fine-tuning histone acetylation in cassava. *Plant Physiology*. **193**: 2232–2247.

144. Zhang, B., He, P., Luo, W., Wang, P., Li, D., Li, B. (2020). A histone deacetylase, GhHDT4D, is positively involved in cotton response to drought stress. *Plant Molecular Biology.* **104**: 67–79.

145. Zhang, H., Lang, Z. and Zhu, J. (2018). Dynamics and function of DNA methylation in plants. *Nature reviews Molecular cell biology*. **19**: 489–506.

146. Zhang, H., Zhang, T. and Chen, L. (2019). Phosphorylation of Histone H2A at Serine 95: A Plant-Specific Mark Involved in Environmental Stress Response. *Frontiers Plant Science*. **10**: 940–963.

147. Zhang, M., Da Silva, J., Yu, Z., Wang, H., Si, C., Zhao, C., He, C. and Duan, J. (2020). Identification of histone deacetylase genes in Dendrobium officinale and their expression profiles under phyto hormone and abiotic stress treatments. *Peer J.* **8**: 10482–10502.

148. Zhang, W., Wang, N., Yang, J., Guo, H., Liu, Z., Zheng, X., Li, S. and Xiang, F. (2020). The salt-induced transcription factor GmMYB84 confers salinity tolerance in soybean. *Plant Science*. **291**: 110326–110356.

149. Zhang, X., Zhu, J.-K., and Song, C. (2007). Epigenetic inheritance in plants. Annual Review of Plant Biology. **58**: 407–429.

150. Zhang, Y., Sun, Z., Jia, J., Du, T., Zhang, N., Tang, Y., Fang, Y. and Fang, D. (2021). Overview of histone modification. *Histone Mutations and Cancer*. **161**: 1–16.

151. Zhao, H., Ge, Z., Zhou, M., Zeng, H., Wei, Y., Liu, G., Yan, Y., Reiter, R.J., He, C. and Shi, H. (2023). Histone deacetylase 9 regulates disease resistance through fine-tuning histone deacetylation of melatonin biosynthetic genes and melatonin accumulation in cassava. *Journal of pineal research*.**74**: 12861–12881.

152. Zhao, T., Zhan, Z. and Jiang, D. (2019). Histone modifications and their regulatory roles in plant development and environmental memory. *Journal of genetics and genomics*. **46**: 467–476.

153. Zheng, M., Lin, J., Liu, X., Chu, W., Li, J., Gao, Y., An, K., Song, W., Xin, M., Yao, Y. and Peng, H. (2021). Histone acetyltransferase TaHAG1 acts as a crucial regulator to strengthen salt tolerance of hexaploid wheat. *Plant Physiology*. **186**: 1951–1969.

154. Zhu, W., Yang, C., Liu, Q., Peng, M., Li, Q., Wang, H., Chen, X., Zhang, B., Feng, P., Chen, T. and Zeng, D. (2023). Integrated analysis of DNA methylome and transcriptome reveals epigenetic regulation of cold tolerance in Litopenaeus vannamei. *International Journal of Molecular Sciences*. **24**: 11573–11592.