

# *Advances in Plant Epigenetic Regulation of Abiotic Stress Response*

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**Abstract:** With global climate change and increasing environmental pollution, plants are facing increasingly severe abiotic stresses, such as drought, salinization and heavy metal pollution. These stresses not only affect plant growth and development, but also pose a threat to agricultural production and ecosystem stability. In order to adapt to these unfavorable environments, plants have evolved a series of complex response mechanisms, among which epigenetic regulation, as an important means of regulation, has gradually attracted the attention of researchers. By modifying gene expression without modifying the DNA sequence, epigenetic control uses a variety of techniques, including DNA methylation, histone modification, and non-coding RNAs (ncRNA). These mechanisms are crucial for plant responses to abiotic stresses and can significantly enhance plant resistance and adaptive capabilities. DNA methylation can enhance plant resistance by controlling the expression of stress response-related genes; histone modification can regulate plant physiological responses by altering the structure of chromatin and affecting the accessibility of genes; and ncRNAs, especially microRNAs and siRNAs, can regulate plant physiological responses by targeting the expression of stress response-related genes. In-depth study of the role of plant epigenetic regulation in abiotic stress response is of great theoretical and practical significance for improving crop resistance and ensuring food security.

**Keywords:** Plant epigenetic regulation, abiotic stress, non-coding RNAs.

## 1. Introduction

Numerous abiotic stressors are present in the natural environment for plants, which mainly include drought, salinization, extreme temperatures and heavy metal pollution. These elements may cause plant mortality in addition to influencing plant growth and development, seriously threatening the stability of ecosystems and the sustainability of agricultural production. Drought directly affects plant photosynthesis, respiration, and nutrient transport by limiting water availability, which in turn leads to growth stagnation, leaf wilting, and yield loss. Salinity stress, on the other hand, alters the osmotic pressure and ionic balance of the soil, inhibits plant root development, affects water and nutrient uptake, and causes physiological disorders. Temperature extremes (e.g., high or low) also challenge plant survival. High temperatures can lead to rupture of cell membranes, denaturation of proteins and inhibition of photosynthesis, while low temperatures may trigger the formation of intracellular ice crystals, damaging cellular structures. Heavy metal contamination interferes with plant physiology and metabolism by accumulating in the plant, leading to toxic reactions.

Plants respond to these abiotic stressors by undergoing a number of intricate physiological and biochemical changes, known as epigenetic regulatory responses in plants. Without altering the DNA sequence, they can change how their genes are expressed, thus responding rapidly and flexibly to environmental changes. In plants, epigenetic regulation is crucial for protein conformation, transcriptional initiation, and the control of hormone and metabolite profiles [1]. The research of plant epigenetics in abiotic stress response systems has advanced significantly in recent years. Through high-throughput sequencing, genome editing, and transcriptome analysis, researchers have explored the epigenetic regulatory mechanisms of plants in response to various abiotic stresses. Gene accessibility can be changed by DNA methylation and histone modification, which subsequently impacts gene transcriptional activity. By focusing on particular mRNAs, non-coding RNAs (ncRNAs), particularly microRNAs and siRNAs, can precisely control gene expression and contribute to plant reactions to abiotic stressors. In-depth study of the role of epigenetic regulation in plant response mechanisms not only helps to understand the physiological adaptation process of plants, but also provides new ideas for agricultural production.

This study will systematically analyze the consequences of various abiotic stressors (e.g., drought, salinity, high temperature, etc.) on plant epigenetic traits, and explore how these stresses may regulate the expression of genes related to stress tolerance by altering the expression patterns of DNA methylation, histone modification, and ncRNAs. Studying the relationship between these will provide us with a more comprehensive perspective and help reveal the complex response mechanisms of plants under adversity.

## 2. Epigenetic Regulatory Mechanisms

### 2.1. DNA Methylation

One of the most crucial processes in epigenetic regulation is DNA methylation, which mainly refers to the addition of methyl (-CH<sub>3</sub>) groups to specific cytosine (C) residues in DNA molecules to form 5-methylcytosine. The CHH, CHG, and CG sequence environments in plants include 5-methylcytosine (5-mC) (where H is any base other than G) [2]. This process usually occurs at CpG dinucleotide sites and affects gene expression and regulation. In addition to being crucial for plant development, DNA methylation also demonstrates a special regulatory role in reaction to abiotic stressors. DNA methylation can repress potentially deleterious genomic elements, such as repetitive sequences and transposon elements (TEs), or It has the ability to transcriptionally control transcription factor expression, indirectly affecting the response of downstream genes, forming a complex regulatory network [2].

It has been shown that under high temperature stress, the RNA-directed DNA methylation (RdDM) pathway affects the transcription of genes close to transposons by altering the methylation state of the DNA or TEs of the transposons, thereby increasing the basal heat tolerance of plants [3]. After exposure to high temperature stress, whole-genome methylation was significantly increased in kale-type oilseed rape, and DNA methylation levels were significantly higher in *Arabidopsis* than in *Arabidopsis* and *Quercus* spp. under extreme heat conditions[3].

In a symmetrical CG environment, DNA methylation is maintained by the methyltransferase MET1. When MET1 is disrupted, CG methylation is lost throughout the genome, and H3K9me<sub>2</sub> and H3K27me<sub>3</sub> are expressed ectopically [2,4]. In several transposon families across the genome, while non-CG methylation and H3K9me<sub>2</sub> reinforce each other through a feedback loop, the repressor mark H3K27me<sub>3</sub> compensates for the absence of DNA hypermethylation/H3K9me<sub>2</sub> [4]. This suggests that changes in DNA methylation do not occur in isolation, but rather interact with other epigenetic modifications in the plant stress response. DNA methylation has a significant impact on chromatin accessibility, and it interacts with histone modifications to generate a complicated code for

transcription and chromatin regulation in the genome [5]. This interaction allows plants to rapidly and efficiently regulate gene expression in response to external stresses under complex environmental conditions.

## 2.2. Histone Modifications

Histone modification is an important component of epigenetic regulation and involves chemical modification of histones, including acetylation, methylation, phosphorylation and ubiquitination. These modifications affect the structure of chromatin and gene expression, as well as alter the conformation of chromatin, which in turn regulates the transcriptional activity of genes. Histones and other proteins interact to encapsulate DNA into chromatin, and DNA is wrapped around histone octamers in eukaryotes [6].

When it comes to how plants react to abiotic stressors, histone changes are essential. It has been shown that some epigenetic regulators, such as acetyltransferases, methyltransferases, deacetylases, and demethylases, mediate methylation and acetylation during high-temperature stress response [7]. Under high-temperature stress, *Arabidopsis thaliana*, which lacks the acetyltransferase GCN5, exhibits severe heat tolerance defects, and heat stress transcription factor 1 (HSF1) in tomato recruits histone acetyltransferase 1 (HAC1), and the interaction between the two regulates the expression of heat-response-related genes [8]. Under salt stress, the kinase GsSnRK1 is activated in wild soybean, which subsequently phosphorylates the histone acetyltransferase GsMYST1, acetylates the transcription start region of COR15B, a gene that the transcription factor GsNAC83 targets downstream, and mediates the epigenetic regulation of the COR gene, thereby promoting soybean resistance to salt stress [9]. Furthermore, it has been demonstrated that altered histone modifications are crucial for the development and regeneration of healing tissues [10].

Dynamic changes in histone modifications enable plants to react quickly to changes in the environment. By regulating the modification status of histones, plants can rapidly initiate or shut down the expression of specific genes under abiotic stresses, thereby regulating physiological processes. The interaction of histone deacetylases (HDACs) and histone acetyltransferases (HATs) is able to regulate the expression of genes related to water use efficiency under drought conditions, thereby increasing plant stress tolerance [11]. Histone modifications also interact with other epigenetic mechanisms to form complex regulatory networks. For example, it has an impact on ncRNA expression, which further controls gene transcription. An in-depth study of the role of histone modifications in plant abiotic stress response not only helps to reveal the molecular mechanisms of plant adaptation to adversity, but also provides new ideas and strategies for plant breeding and agricultural production.

## 2.3. NcRNA

ncRNAs are a class of regulatory RNAs consisting of microRNAs (miRNAs), small interfering RNAs (siRNAs), long-chain noncoding RNAs (lncRNAs), and circular RNAs (circRNAs). The key roles played by the first three in plant adversity stress response will be highlighted below.

### 2.3.1. microRNAs

A significant part of plant epigenetic control is played by miRNAs, a type of tiny ncRNA molecules that range in length from 21 to 24 nucleotides. miRNAs mainly form RNA-induced silencing complexes (RISCs) together with Argonaute proteins (AGO1), and miRISCs have the ability to initiate phasiRNA synthesis, mediate translational repression, or guide transcriptional splitting, or even direct DNA methylation [12]. miRNA biosynthesis involves three phases: transcription, processing, and maturation. RNA polymerase II first transcribes miRNAs to produce primary

miRNAs (pri-miRNAs), which are subsequently broken down by the Dicer enzyme in the nucleus to produce pre-miRNAs. In the cytoplasm, miRNAs undergo additional processing and maturation to become functional miRNAs.

miRNAs are essential regulators of plants' responses to abiotic stress, they modulating the expression of genes associated with stress response. Under cold stress, in *Arabidopsis thaliana*, miR397 targets the cold signaling pathway's LACs (laccases) and CKB3 (casein kinase II subunit Beta 3) to produce elevated levels of CBF and COR, thereby increasing stress tolerance [13]. Under salt stress, barley miR408 targets the genes phosphate transporter (PT) gene NtPT2, and the genes encoding abscisic acid receptor and SnRK2 proteins, NtPYL2 and NtSAPK3, to enhance phosphorus uptake [14]. miRNAs are also involved in the regulation of physiological processes such as antioxidant response, ion homeostasis and osmotic adjustment in plants, which enhances the plant's environmental stress tolerance.

Studies have shown that miRNAs can form a complex regulatory network by regulating transcription factors, signaling pathways and other key nodes. miR166 targets HD-ZIP (homology domain leucine zipper), miR167 targets ARF, miR168 targets AGO, miR171 targets SCL, and miR396 targets GRF (growth regulator factor), regulating plant growth and development, and also involved in abiotic stress adaptation [15]. This multiple regulatory mechanism makes miRNAs play an indispensable role in plant adaptation to environmental changes. Non-conserved miRNAs are selectively expressed in different plants, and little is known about their specific expression mechanisms in non-medicinal plants and agricultural crops, which are relatively limited at present. However, with the development of new biotechnology, microRNAs, as an important part of plant epigenetic regulation, will be further deciphered and improved in their mechanism and regulatory network, contributing to the cultivation of superior varieties.

### 2.3.2. siRNAs

siRNAs are an important class of ncRNAs, usually generated from long double-stranded RNAs (dsRNAs) by cleavage of Dicer enzyme, which is generally 20-25 nucleotides in length, and can be mainly subclassified into heterochromatic small interfering RNAs (hc-siRNAs) and secondary siRNAs. The most prevalent siRNAs in plants are hc-siRNAs [12]. siRNAs, accounting for more than 90% of siRNAs in *Arabidopsis* [12]. While secondary RNAs include phasic secondary siRNAs (phasiRNAs), trans siRNAs (tasiRNAs), and epigenetically activated RNAs (easiRNAs), etc. hc-siRNAs regulate the stability of the plant genome and gene expression through the RdDM pathway, and their most typical biological function is to silence transposons to maintain genomic integrity, and it is also involved in the regulation of genomic imprinting and interploidal hybridization barriers; secondary small interfering RNAs are able to mediate transcriptional cleavage of different genes, mediate trans-target mRNAs, or split their own precursors [12]. However, this fraction of RNAs is not well understood, and the molecular functions of many secondary small interfering RNAs are not well characterized.

The production and function of siRNA are regulated under abiotic stress conditions. Under salt stress and drought stress, the level of dsRNA in plant cells may significantly increase, which in turn promotes siRNA synthesis. These siRNAs are able to target genes related to stress response and regulate their expression levels, thus helping plants to adapt to unfavorable environments. In *Arabidopsis thaliana*, TAS1 siRNA was able to target heat-responsive genes induced by HTT1 and HTT2 to regulate its heat tolerance [16], and TAS4 siRNA was able to target the MYB family of transcription factors to regulate anthocyanin synthesis and trichome development [17]. Upon pathogen attack or abiotic stress, siRNAs are able to propagate within the plant and activate resistance responses in distal tissues. This transcellular signaling mechanism enables plants to respond rapidly in the face of environmental changes and enhances overall survivability.

### 2.3.3. lncRNAs

lncRNAs are a subset of RNA molecules more than 200 nucleotides in length, which, despite not encoding proteins, they are crucial regulators of Plant ontogeny and morphogenesis as well as how plants react to abiotic stressors. In the last few years, lncRNAs have been found to play key roles in plant epigenetic regulation, especially in regulating gene expression and chromatin state.

Through their cis and trans mechanisms of action, lncRNAs control the expression of both proximal and distal protein-coding genes. lncRNAs and mRNAs may interact with each other by competing with the same miRNAs, or miRNAs and lncRNAs can interact with each other to regulate the function of the same mRNAs. In cabbage (*B. rapa*), lncRNAs may regulate target genes or heat-responsive genes (heat-shock proteins HSPs, heat stress factors HSFs, THE, and DREB2A) as a result of competition between lncRNAs and protein-coding genes for binding to miRNAs (bra-miR159a or bra-miR172a) [18]. lncRNAs can also act as regulators to influence the expression of genes related to anthocyanin biosynthesis pathway [19], which results in strong antioxidant activity of plants in response to different abiotic stresses. During seed germination in *Arabidopsis thaliana*, AtR8 lncRNA expression responds to salt stress, and AtR8 lncRNA deletion inhibits seed germination [20].

Although research on lncRNAs is still in its infancy, existing studies have shown that the role of lncRNAs in plant epigenetic regulation cannot be ignored. Future studies should further reveal the specific functions of lncRNAs and their regulatory networks to better understand how plants adapt to complex environmental changes through epigenetic mechanisms. This not only provides new perspectives for basic science research but also offers potential application prospects for agricultural production, especially in improving crop resistance and adaptation.

## 3. Applications

### 3.1. Genetic Improvement and Applications in Agriculture

Currently, the global food situation is critical. The number of hungry people is increasing, food insecurity is serious and regional differences are significant. According to reports issued by the Food and Agriculture Organization of the United Nations and other agencies, as of 2023, about 733 million people worldwide will face hunger, and the situation in Africa is particularly serious, with 58 percent of the population in a situation of moderate or severe food insecurity. Among them, climate extremes are one of the major influences on global food problems. Genetic improvement is an important tool to enhance crop resilience, and plant epigenetic regulation provides new perspectives and approaches to this process. Through in-depth study of plant epigenetic mechanisms, scientists are able to identify key genes associated with abiotic stress response and utilize this information for precise genetic improvement.

In crop breeding, epigenetic variation can be used to improve crop responsiveness to environmental changes, thereby increasing yield and quality. Through the screening of epigenetic markers, breeders are able to identify individuals with excellent resistance at an early stage, thus accelerating the breeding process. Meanwhile, by improving the genetic characteristics of crops through molecular breeding and gene editing technologies, their yield, quality, and resistance will be significantly improved and adapted to climate change, thus realizing high-yield, high-efficiency, and sustainable development of agriculture. In the future, with deeper research and technological advances, plant genetic breeding will become more precise and efficient, and new technologies such as gene editing and molecular marker-assisted selection will be more widely used.



### 3.2. Improvement of Plant Resilience and Forest Tree Breeding

With the development of human activities, especially the high energy consumption and emissions in the process of industrialization and urbanization, there has been global warming, loss of biodiversity, water scarcity and soil pollution. People are facing unprecedented challenges in global environmental issues. In the process of harmonizing the sustainable development of man and nature, forests are key players and important guardians in maintaining soil and water, nourishing water, regulating climate and purifying pollution. However, forest fires, pests and diseases, and climate change are posing multiple threats to global forests. Improving plant resilience is an important strategy to cope with global climate change and environmental stress, especially critical in forest tree breeding. Combining the technical means of epigenetic regulation provides new ideas and methods for forest tree breeding.

Epigenetic regulation can enhance plant adaptation to adversity without introducing exogenous genes by modifying the expression of genes without modifying the DNA sequence. The combination of genomics and transcriptomics research can deeply reveal the epigenetic regulation mechanism of forest trees under abiotic stress and provide more precise targets for breeding. On the gene expression scale, by regulating the expression of genes associated with wood quality, disease resistance, etc., better quality forest tree varieties can be bred. During wood formation, the synergistic effects of transcription factors and histone modifications regulate the development of the vascular formation layer, which in turn affects wood quality and growth rate. Research findings on epigenetic regulation also continue to guide molecular breeding.

## 4. Conclusion

Recently, in light of recent molecular biology advancements, the realm of plant epigenetics has witnessed significant strides. This study reveals the importance of epigenetic mechanisms in plant adaptation to adverse environments by exploring the role of plant epigenetic regulation in abiotic stress response. Studies have illustrated that by managing the genes that respond to environmental stresses, epigenetic controls like DNA methylation, histone modifications, and ncRNAs can strengthen a plant's ability to withstand adverse conditions. These mechanisms are essential not only for the endurance of plants in harsh conditions, but also provide fresh insights into how plants react to intricate ecological shifts. Although the secrets of epigenetic regulation are being revealed, the specific mechanisms of action under different abiotic stress conditions, especially the dynamics of epigenetic marks and their effects on gene expression, still need to be further explored. How to effectively utilize the results of epigenetic regulation in plant breeding and agricultural production remains an important direction for future research.

Going forward, as technologies like genome sequencing, bioinformatics, and epigenomics continue to advance, it is possible to study non-model plants (agricultural crops and forestry trees), and we are expected to reveal the complex network of epigenetic regulation in plants and their specific roles in abiotic stress response in a more in-depth manner.

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