WHIOCE PUBLISHING PTE. LTD.
PROVIDING
FIRST-CLASS SCIENTIFIC INFORMATION
FOR TOP SCHOLARS

2025 Volume 3, Issue 10
Lecture Notes in Education, Arts, Management and Social Science
ISSN(Online): 2705-053X

The Mechanism of Epigenetic Modifications in Long-term Adaptation to Salt Stress

Yaxin Tan*
University of California, Riverside, California 92521, USA

**Author to whom correspondence should be addressed.*

Abstract: *Objective:* This paper aims to investigate the mechanism by which epigenetic modifications play a role in the formation and maintenance of salt tolerance under long-term salt stress, and to clarify the regulatory network played by DNA methylation, histone modification, and non-coding RNA during long-term adaptation. *Methods:* A long-term salt stress treatment model was established, and the subjects were divided into a control group, a long-term salt stress group, and a recovery group. The recovery group was used to evaluate epigenetic memory. Salt tolerance phenotypes were evaluated by growth status, ionic homeostasis (Na^+/K^+), osmotic regulators, oxidative stress indicators, DNA methylation, histone modification, and non-coding RNA expression changes were detected by bisulfite sequencing, ChIP-qPCR, and transcriptome sequencing, and their causal effects were verified by epigenetic-related inhibitors or key enzyme interventions. *Results:* Long-term salt stress can improve the salt-tolerant phenotype, namely reduced growth inhibition, decreased Na^+ accumulation, and enhanced antioxidant capacity. Remodeling of genome-wide methylation patterns, reduced methylation levels of salt-tolerant gene promoters and upregulated transcription; At the same time, there is an enhancement of active histone markers (H3K4me3, H3K9ac), which continuously activates salt-tolerant genes. The non-coding RNA network was also reconstructed, with some mirnas and lncrnas performing fine regulation by targeting genes related to ion transport and ABA signaling. Blocking DNA methylation or histone deacetylation weakens the salt-tolerant phenotype and reduces the expression of related genes. *Conclusion:* Long-term salt stress adaptation is a co-reprogramming of multiple levels of epigenetic modifications, where DNA methylation, histone modification, and non-coding RNA together continuously activate salt-tolerant genes and potentially form epigenetic memory, providing potential targets for salt tolerance improvement and salt-related injury intervention.

Keywords: Epigenetic modifications; Long-term adaptation to salt stress; Mechanism of action

Online publication: November 26, 2025

1. Introduction

Salt stress is one of the common environmental stress factors, which can cause cellular water imbalance, disruption of Na^+/K^+ homeostasis and excessive accumulation of reactive oxygen species (ROS) due to osmotic stress and ionic toxicity, leading to growth retardation and even death[1]. Unlike short-term salt stress, long-term salt stress generally causes the body to develop a relatively stable adaptive phenotype, showing strong tolerance when exposed to salt stress for a long time or repeatedly. But long-term adaptation is not just about transient changes in signaling pathways, but more about the

continuous remodeling of gene expression regulation.

Recent studies have found that epigenetic modifications play a key role in the formation of environmental stress memory, such as DNA methylation rearrangement, histone modification changes, and non-coding RNA-mediated transcriptional regulation, which keep salt tolerance-related genes activated or silenced for a long time by influencing chromatin openness and transcriptional readiness, thus maintaining an adaptive state[2]. Therefore, this paper intends to take the role of epigenetic modifications in long-term salt stress adaptation as the research object to provide a basis for understanding the formation of salt stress tolerance and subsequent targeted interventions.

2. Materials and methods

2.1. Research subjects and groups

Model organisms with the same growth status and developmental stage (represented by Arabidopsis thaliana) were selected as experimental materials in this study. All samples were cultured under the same light cycle, temperature and humidity, and nutritional conditions to minimize the influence of environmental differences on the results. The plants were randomly grouped into the control group, the long-term salt stress group, and the recovery group. The control group was continuously cultivated under normal culture conditions without salt treatment; The long-term salt stress group received continuous salt treatment after stable growth to simulate the environment of chronic salt stress and induce the formation of adaptive phenotypes; The recovery group was transferred to a salt-free environment for continued culture after long-term salt treatment to evaluate whether the salt-tolerant phenotype and related epigenetic markers could be maintained after the stress was relieved and to determine the existence of epigenetic memory. At least three biological replicates were set for each group.

2.2. Long-term salt stress treatment regimens

In order to simulate the chronic stress conditions of persistent salinization in the natural environment and to form a stable salt-tolerant adaptive phenotype in the study subjects, a long-term salt stress treatment model was established in this study. All experimental materials were subjected to salt treatment only after they had germinated and entered the stable growth phase to ensure that the samples had a consistent basal physiological state[2]. Salt stress used sodium chloride (NaCl) as the primary stress source and was carried out in a step-up manner over a long period of time to avoid acute injury or non-specific death caused by short-term high salt, thus more accurately reflecting the process of long-term adaptation.

Salt stress is not applied until the plants have reached the 4-leaf true leaf stage under standard culture conditions. Pre-adaptation with 50mM NaCl for 3 days to give the plants an initial buffering capacity to changes in osmotic pressure; After that, the salt concentration was gradually increased by 50mM every 3 days until the final long-term treatment concentration was 150mM (or set to 150mM-200mM depending on the tolerance range of the study subjects). After reaching the target concentration, maintain a stable salt treatment environment and continue the treatment for 4 to 6 weeks to create a long-term salt stress model. Daily observation of plant growth status, leaf wilting degree, yellowing ratio, root growth rate, overall development rate, survival rate and morphological indicators[3]. All treatments were carried out under the same light cycle (16 hours of light, 8 hours of dark), temperature (about 22 ° C), and relative humidity (about 60%) to reduce systemic bias caused by non-salt factors.

In order to improve the repeatability and physiological consistency of the long-term salt stress model, uniform irrigation volume, uniform salt solution concentration, and uniform treatment time were adopted during the salt treatment. The salt solution was changed every 2 to 3 days to prevent evaporation from causing an increase in salt concentration or a change in the ionic ratio of the solution. At the same time, during the long-term salt stress maintenance phase, changes in the electrical conductivity (EC value) of the culture medium or irrigation fluid are regularly monitored as an indirect quality control indicator of salt stress intensity to ensure the stability of different batches of treatment. For soil culture systems, use the same substrate ratio, pot specifications, and weighing method to control the amount of irrigation so that

each sample receives approximately the same salt input load to prevent individual bias caused by differences in local salt accumulation.

In order to understand the process of salt-tolerant phenotypes and epigenetic changes under long-term salt stress, stratified sampling was conducted over different time periods in this paper. The sampling time points were: before salt treatment (T0), week 1 after reaching the target salt concentration (T1), week 4 (T4), week 6 (T6) (or week 8 depending on the treatment cycle). Each time, the same developmental stage of leaf or root tissue was taken, rapidly frozen in liquid nitrogen and stored at -80 ° C for subsequent DNA methylation, histone modification, and transcriptome detection.

To minimize the differences in gene expression caused by circadian rhythms, all samples were taken at fixed times of the same photoperiod (10 a.m. to 12 p.m.). And at each time point, more than three biological repeats were set up, and each repeat was combined with multiple plants to reduce the impact of individual plant fluctuations on omics results.

A recovery group was set up in this study to test whether the salt-tolerant state formed by long-term salt stress was persistent and whether epigenetic memory was retained after relief. In the recovery group, after long-term salt stress treatment, the plants were moved to salt-free conditions and continued to be cultivated for 7-14 days[4]. No salt stimulation was added during the recovery period, and growth recovery, ionic homeostasis, and oxidative stress-related indicators were continued to be monitored. Recovery endpoint sampling (R7/R14) was used to determine whether salt-tolerant phenotypes still existed in a salt-free environment and whether epigenetic markers (reduced promoter methylation or enriched active histone markers) could still be detected, in order to determine whether long-term salt adaptation formed epigenetic memory.

In addition, to distinguish the difference between long-term adaptation and Short-term response, a short-term salt stress control group could be set up in this study, and samples were taken after treatment at the same salt concentration for 24 to 72 hours. By comparing the epigenetic differences between the short-term and long-term groups, we can identify the characteristics of DNA methylation remodeling, histone modification stabilization, and non-coding RNA network remodeling that are specific to the long-term adaptation stage, preventing the misjudgment of acute response signals as long-term adaptation mechanisms.

Throughout the long-term salt treatment, in order to eliminate the influence of factors other than nutrient deficiency or osmotic pressure on the results, all culture systems followed the principle of isotonic control, that is, the control group used the same ion background culture medium, but no NaCl was added; If the experiment requires a distinction between ionic toxicity and osmotic effects, an isotonic mannitol treatment group can be set up for verification[5]. All the treatment parameters are optimized based on the pre-experiment, which can significantly induce the salt stress response without causing massive deaths, ensuring a reliable sample source and statistical power for subsequent multi-omics detection and mechanism validation experiments.

2.3. Phenotypic evaluation indicators

To evaluate the long-term salt stress adaptation phenotype, this paper examines four aspects: growth, ionic homeostasis, osmotic regulation, and oxidative stress. Growth indicators such as survival rate, plant height, fresh weight/dry weight, and root length were recorded; The content of Na^+ and K^+ was determined by flame photometry or ICP and the Na^+/K^+ ratio was calculated to reflect the ion balance; Osmotic regulation was evaluated using proline (ninhydrin method), soluble sugar (anthrone method), and relative water content; ROS accumulation, MDA content and SOD, POD, CAT activity were detected to evaluate oxidative damage and antioxidant levels. Each indicator was sampled at fixed times for at least three biological replicates.

2.4. Epigenetic testing

To investigate epigenetic remodeling under long-term salt stress, DNA methylation, histone modification, and non-coding RNA were detected in this study. DNA methylation was sequenced using bisulfite sequencing or targeted BS-PCR to identify differentially methylated regions and annotate related gene pathways; Histone modifications were detected by

ChIP-qPCR or ChIP-seq for enrichment of markers such as H3K4me3, H3K9ac, and H3K27me3 in key salt-tolerant gene promoter regions; Non-coding RNA was screened for differentially expressed molecules by small RNA sequencing and lncRNA analysis, and the ncrNA-target gene regulatory network was constructed by combining target gene prediction and RT-qPCR verification.

2.5. Key factor intervention/validation experiments

Pharmacological inhibition and functional perturbation were used to examine the causal relationship between epigenetic modifications and long-term salt tolerance.

The methylation process was intervened with DNA methyltransferase inhibitors (5-aza class) to observe salt-tolerant phenotypes and changes in methylation and expression of key genes; HDAC inhibitors (TSA) were used to regulate histone acetylation levels to detect H3K9ac enrichment and sustained activation of salt toleration-related genes. Validation is carried out in combination with knockdown or mutants of key epigenetic enzyme genes if necessary. Simultaneous detection of Na^+/K^+, ROS, and antioxidant indicators after intervention to evaluate whether salt tolerance has decreased.

2.6. Statistical analysis

All experiments had at least three biological replicates, and the data were expressed as mean ± standard deviation. Normality and homogeneity of variance tests were performed before statistics. Independent sample t-tests were used for comparisons between two groups when conditions were met, and one-way analysis of variance with multiple corrections (Tukey or Bonferroni) was used for comparisons among multiple groups. Non-normal data were tested using the Mann-Whitney U or Kruskal-Wallis test. Omics differences were screened using a set threshold, FDR correction method, and pathway changes were evaluated using enrichment analysis.

Correlation analysis was conducted using Pearson or Spearman to evaluate the relationship between methylation/histone modification and gene expression. $P<0.05$ was considered statistically significant.

3. Results

3.1. Significant improvement in long-term salt stress

After prolonged salt stress treatment, the experimental materials developed a stable salt-tolerant adaptation phenotype. The long-term salt stress group showed significantly higher survival rates, reduced growth inhibition, decreased leaf yellowing and wilting, and the root length and lateral root development remained largely intact in the prolonged high-salt environment. Ion homeostasis showed a decrease in sodium ion accumulation levels, an increase in potassium ion retention capacity, and a significant reduction in the sodium-potassium ratio. The accumulation of ROS in oxidative stress indicators was inhibited, MDA content decreased, and the activities of SOD, POD, and CAT increased, indicating that the antioxidant system was strengthened under long-term salt stress. The recovery group still maintained partial salt tolerance after salt-free culture, indicating the existence of an adaptive regulatory basis that can be maintained.

3.2. The DNA methylation profile was remodeled under long-term salt stress

The genome-wide DNA methylation pattern changes significantly under long-term salt stress, with differentially methylated regions (DMRs) distributed in both promoter and genome regions and pathway specific enrichment. The methylation levels in the promoter regions of key genes related to ion transport, osmotic regulation, and antioxidation decreased, while the transcriptional levels increased, indicating that demethylation is involved in the sustained activation of salt-tolerant genes. Some genes related to growth promotion and energy expenditure showed elevated promoter methylation and decreased expression, indicating resource allocation and growth trade-offs during long-term salt adaptation. Some DMRs were still detectable in the recovery group, indicating that DNA methylation remodeling was stable and might be involved in the maintenance of epigenetic memory.

3.3. Histone modification changes and salt tolerance gene activation

Under long-term salt stress, there is a significant rearrangement of histone modification profiles, and active markers and inhibitory markers show different enrichment in different gene sets. ChIP detection showed significantly increased levels of H3K4me3 and H3K9ac in the promoter region of the salt-tolerant core gene, consistent with the upward trend of mRNA expression, indicating that chromatin opening and enhanced transcriptional readiness state prompted its sustained expression. In the gene region associated with cell proliferation and biomass accumulation, H3K27me3 enrichment was enhanced and transcription was inhibited, demonstrating inhibitory regulation of non-essential growth pathways under long-term stress. The enrichment of active modifications in the recovery group was improved to some extent, indicating that histone modifications are involved in the continuous regulation of the salt-tolerant phenotype.

3.4. The non-coding RNA network is involved in the fine-tuning of the salt tolerance mechanism

Long-term salt stress can significantly alter the expression profile of non-coding Rnas. Differentially expressed mirnas and lncrnas together form a regulatory network with biological orientation. A multi-omics synthesis analysis revealed that the partially upregulated miRNA was negatively correlated with the expression of ion transportation-related target genes, suggesting that it regulates ion homeostasis by restricting Na^+ absorption or enhancing ion effloresection pathways. Some lncrnas were elevated after prolonged salt treatment and positively correlated with genes related to stress response and ABA signaling, suggesting that they might be transcriptional regulatory platforms or recruit chromatin modification complexes to influence the expression status of target genes. Abnormal expression of some ncrnas was detected in the recovery group, indicating that it can be involved in the stable maintenance of long-term adaptation and is associated with epigenetic memory formation.

3.5. Epigenetic intervention validation: blocking key modifications weakens salt tolerance

Epigenetic pathway intervention significantly affects the salt-tolerant phenotype induced by long-term salt stress. After treatment with DNA methylation inhibitors, the original growth dominance and survival rate of the long-term salt stress group were weakened, the Na^+/K^+ ratio increased, the content of ROS and MDA increased, and the activity of antioxidant enzymes decreased. After histone deacetylation regulation was blocked, abnormal changes occurred in the acetylation levels in the promoter regions of key salt-tolerant genes, and the degree of salt-tolerant transcriptional activation decreased, phenotypically showing increased growth inhibition and ionic homeostasis imbalance. From the above results, it can be seen that DNA methylation and histone modification are not concomitants of stress, but necessary regulators formed and maintained through long-term adaptation, providing experimental evidence for the establishment of causal chains.

4. Discussion

Long-term salt stress adaptation is a shift from a short-term stress response to a stable phenotype, mainly a continuous reprogramming of the state of gene expression. The results show that DNA methylation remodeling, histone modification rearrangement, and changes in the non-coding RNA network are mutually coupled. DNA methylation regulates the long-term expression of salt-tolerant genes by altering the availability of promoters, the enrichment of histone active markers enhances the transcriptional readiness state and improves the ability of sustained activation, and inhibitory markers participate in the down-regulation of growth pathways to reduce energy consumption.

Non-coding Rnas provide a delicate regulatory interface between signaling pathways and epigenetics, strengthening ionic homeostasis and antioxidant defense.

The recovery group retained some molecular markers indicating epigenetic memory, which could explain the mechanism by which long-term salt tolerance remains stable and also provide a theoretical basis for improving salt tolerance through epigenetic factors.

Disclosure statement

The author declares no conflict of interest.

References

[1] Cheng CX, Zhao JZ, Zhao YH, 2025, Effects of Low-temperature Stratification Treatment on Seed Germination of Goji Berries under Sodium Salt Stress. Journal of Central South University of Forestry and Technology, 1-9.

[2] Yu YJ, Yang X, Ma R, et al., 2025, Analysis of Changes in Endogenous Plant Hormone Content and Related Gene Expression in Oats under Saline-Alkali Stress. Grassland Science, 1-23.

[3] Li HY, Yao T, Bai J, et al., 2025, Effects of Inoculation of Three Beneficial Bacteria on Growth and Photosynthetic Characteristics of Alfalfa under Salt Stress. Pratology Science, 1-16.

[4] Li HY, Liao FZ, Wang F, et al., 2020, Physiological Responses and Salt Tolerance Mechanisms of Sweet Cherry Rootstock and Spike Combination under Salt Stress. Acta Agriculturae Sinica, 40(06): 110-116.

[5] Wang SM, Hu R, Liu JJ, et al., 2020, Effects of Salt Stress on Physiological and Biochemical Characteristics of Maize Seedlings with Different Genetic Backgrounds. Anhui Agricultural Sciences, 53(24): 41-44+52.