

633.511:631.527.53:577.2:581.133.1

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PROSPECTS FOR THE USE OF GENETIC ENGINEERING METHODS TO ENHANCE SALINITY TOLERANCE IN COTTON

Abstract. *Salinity refers to the accumulation of water-soluble mineral salts in the soil. Based on the chemical composition of these salts, salinities can be categorized as soda, soda sulfate, sulfate, sulfate chloride, chloride sulfate, and chloride. These types of salinity adversely affect plant productivity, necessitating the development of new varieties resistant to salinity. Numerous miRNAs and genes that respond to salinity stress have been identified, offering potential targets for breeding and genetic engineering. This article discusses these miRNAs and genes, highlighting their roles in plant salinity tolerance.*

Key words: *salinity, RNA interference (RNAi), CRISPR/Cas9, ST7 gene, GhNHX1, GhFSD1, BRX (BREVIS RADIX) gene family, miRNA*

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**ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ МЕТОДОВ ГЕННОЙ
ИНЖЕНЕРИИ ДЛЯ ПОВЫШЕНИЯ УСТОЙЧИВОСТИ
ХЛОПЧАТНИКА К ЗАСОЛЕНИЮ**

***Аннотация.** Засоление – это процесс накопления водорастворимых минеральных солей в почве. По химическому составу соли различают содовые, натронно-сульфатные, сульфатные, сульфатно-хлоридные, хлоридно-сульфатные и хлоридные минерализации. Эти засоления отрицательно влияют на продуктивность растений. Это требует создания новых сортов, устойчивых к засолению. Идентифицировано несколько микроРНК и генов, которые реагируют на стресс, вызванный засолением. В этой статье они обсуждаются.*

Ключевые слова: засоление, РНК интерференция (RNAi), CRISPR/Cas9, ген ST7, ген GhNHX1, GhFSD1, BRX (BREVIS RADIX) семейство генов, микроРНК

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G‘O‘ZA O‘SIMLIGINING SHO‘RLANISHGA CHIDAMLILIGINI OSHIRISHDA GEN MUHANDISLIGI USULLARIDAN FOYDALANISH ISTIQBOLLARI

Annotatsiya. Sho‘rlanish-suvda eriydigan mineral tuzlarning tuproqda to‘planish jarayoni. Tuzlarning kimyoviy tarkibiga ko‘ra sodali, sodasulfatli, sulfatli, sulfatxloridli, xloridsulfatli, xloridli sho‘rlanishlar bo‘ladi. Bu sho‘rlanishlar o‘simliklarning hosildorligiga salbiy ta‘sir qiladi. Bu esa, sho‘rlanishga chidamli bo‘lgan yangi navlarni yaratishni taqozo etadi. Sho‘rlanish stressiga javob beruvchi bir qancha miRNK va genlar aniqlangan. Ushbu maqolada ular haqida to‘xtalib o‘tilgan.

Kalit so‘zlar: *sho‘rlanish, RNK interferensiyasi (RNAi), CRISPR/Cas9, ST7 geni, GhNHX1 geni, GhFSD1, BRX (BREVIS RADIX) genlar oilasi, miRNK.*

Introduction. Soil salinization is one of the most significant abiotic stressors that adversely affect the growth and productivity of agricultural crops. It is estimated that approximately 20–33% of the world’s irrigated farmland is impacted by salinity, leading to substantial crop yield losses annually. The excessive accumulation of salts, particularly sodium (Na^+) and chloride (Cl^-) ions, in the soil disrupts the ionic homeostasis in plant tissues, thereby impairing water uptake, nutrient balance, and cellular metabolism, ultimately inhibiting plant growth and development.

The intensification of soil salinization is driven by multiple interrelated factors, including global climate change, unsustainable land management practices, and inefficient irrigation systems. These challenges underscore the urgency of developing crop varieties with enhanced tolerance to saline conditions. However, conventional breeding methods for salinity tolerance are time-consuming, labor-intensive, and often constrained by the polygenic nature of the trait and the complexity of environmental interactions.

Advances in modern biotechnology offer promising alternatives to traditional breeding. Genetic engineering techniques, such as RNA interference (RNAi), transgenic overexpression, and CRISPR/Cas9-based genome editing, have emerged as powerful tools for introducing or modifying specific genes associated with salinity tolerance. These technologies enable targeted manipulation of genes involved in key physiological and biochemical pathways, facilitating the development of stress-resilient cultivars with greater precision and efficiency.

Genetic Approaches to Enhance Salt Tolerance in Cotton

Cotton (*Gossypium* spp.) is one of the most economically important industrial crops globally, primarily cultivated for its natural fiber. In addition to its role in the textile industry, cotton serves as a key raw material for various sectors, including pharmaceuticals, light industry, and agriculture. It accounts for approximately 35% of global natural fiber consumption [1]. However, cotton is

highly susceptible to abiotic stress factors, particularly soil salinity, which can cause yield losses of up to 50% under severe conditions [1,2].

Soil salinity disrupts plant physiological and metabolic processes by inducing osmotic stress and ion toxicity, particularly through the accumulation of sodium (Na^+) ions in plant tissues. This leads to the inhibition of seed germination, reduced biomass accumulation, and impaired reproductive development. In an effort to improve salinity tolerance in cotton, researchers have utilized genes from halophytic plants. For instance, overexpression of the *ST7* gene from *Thellungiella halophila* in cotton resulted in improved seed germination and early seedling growth under NaCl-induced stress. The transgenic cotton lines also showed higher yields and moderately increased antioxidant activity compared to non-transgenic controls [1].

Salinity tolerance is a complex quantitative trait governed by both physiological and genetic mechanisms. One of the most important gene families involved in this process encodes Na^+/H^+ antiporters. These are membrane-localized proteins found in the plasma membrane and tonoplast that help regulate intracellular ion homeostasis. By transporting excess Na^+ into the vacuole or out of the cytoplasm, they maintain cellular osmotic balance and prevent the toxic effects of salt stress. Numerous studies have demonstrated that overexpression of Na^+/H^+ antiporter genes from other species can enhance salt tolerance in model plants. For example, genes derived from *Reaumuria trigyna*, *Vigna radiata*, and *Triticum aestivum* have been functionally expressed in *Arabidopsis thaliana* using in planta transformation methods, leading to significantly improved tolerance to saline conditions [2].

In addition to plant-derived genes, microbial genes have also shown great promise for improving abiotic stress tolerance. A notable example is the K2-NhaD gene, which encodes a bacterial Na^+/H^+ antiporter isolated from *Alkalimonas amylolytica*, a halotolerant bacterium found in the saline Keke Lake of Xinjiang Province, China. When this gene was overexpressed in cotton, the resulting transgenic plants exhibited significantly enhanced tolerance to both salinity and

drought. These plants also produced more seeds compared to non-transgenic controls under stress conditions, demonstrating the potential of microbial genes in developing stress-resilient cotton cultivars [3].

Molecular Regulation of Ion Transport and Oxidative Stress Response in Cotton Under Salinity Stress. Structural analyses of the NHX (Na^+/H^+ exchanger) gene family in three cultivated cotton species—*Gossypium arboreum*, *G. raimondii*, and *G. hirsutum*—have revealed a complex genomic architecture, characterized by the presence of numerous introns [4]. This intron-rich structure facilitates alternative splicing, resulting in the generation of diverse transcript isoforms. Such transcriptional plasticity enhances the plant's ability to respond to varying concentrations of soil salinity. Functional studies have demonstrated that knockout of the *GhNHX1* gene increases seed sensitivity to elevated salt concentrations, thereby highlighting *GhNHX1* as a key candidate gene for engineering salt-tolerant cotton genotypes [4].

Despite cotton's reputation as a relatively salt-tolerant crop, exposure to high salinity levels can lead to excessive production of reactive oxygen species (ROS), such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-), which disrupt cellular redox homeostasis [5,6]. ROS act as signaling molecules during stress perception but become cytotoxic at elevated levels, causing damage to nucleic acids, oxidation of proteins, and lipid peroxidation, ultimately impairing plant growth and productivity [7].

Recent transcriptomic and small RNA sequencing studies have identified numerous microRNAs (miRNAs) involved in abiotic stress responses. For instance, Xie et al. (2015) identified 337 miRNAs—including 289 known and 48 novel miRNAs—that are differentially expressed under drought and salinity stress conditions in cotton [6]. These miRNAs participate in post-transcriptional regulation of stress-responsive genes.

A specific regulatory module involving *GhFSD1* (iron superoxide dismutase gene) and miRNA *ghr-miR414* has been implicated in the salinity stress response [5]. *GhFSD1* is critical for detoxifying superoxide radicals generated under stress

conditions. Silencing of *GhFSD1* leads to a salt-sensitive phenotype in cotton, while overexpression of *ghr-miR414c* represses *GhFSD1* expression, resulting in a phenotype similar to *GhFSD1*-knockdown plants. This inverse regulatory relationship suggests that *ghr-miR414c* negatively modulates salinity tolerance by targeting *GhFSD1*. These findings open new avenues for the development of miRNA-based genetic engineering strategies to enhance stress resilience in cotton [5].

Role of BRX and AlkB Gene Families in Cotton Salinity Tolerance. Wei et al. (2024) identified members of the BREVIS RADIX (BRX) gene family in three cotton species: 12 genes in *Gossypium hirsutum*, and 6 genes each in *G. raimondii* and *G. arboreum* [8]. Among these, *GhBRX.1*, *GhBRX.2*, and *GhBRXL4.3* exhibited strong expression in roots, with *GhBRXL4.3* also showing significant expression in flowers. Functional analyses of BRX-silenced cotton plants under salinity stress revealed a reduction in the activities of key antioxidant enzymes—including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT)—as well as a decrease in chlorophyll content. These findings suggest that BRX genes may play a crucial role in mediating the cotton plant's defense responses to salt stress by regulating oxidative stress pathways [8].

The alpha-ketoglutarate-dependent dioxygenase (AlkB) gene family is well-established as an important regulator of plant growth, stress sensing, and response mechanisms; however, its function in cotton remains poorly characterized. Comparative genomic studies have identified 40 AlkB homologs in both cotton and *Arabidopsis thaliana*. Notably, suppression of the *GhALKBH10* gene in cotton resulted in enhanced antioxidant capacity and reduced cytosolic Na⁺ ion accumulation, thereby improving salt tolerance [9]. These results indicate that *GhALKBH10* acts as a negative regulator of salinity tolerance, modulating ion homeostasis and oxidative stress responses under saline conditions [9].

Conclusion. Several genes are involved in stress perception and response in plants. Salt tolerance is a complex trait regulated both genetically and

physiologically. Research conducted by scientists has identified several miRNAs and candidate genes related to salt stress in cotton.

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