



MINI REVIEW ARTICLE

A review of approaches to enhance salt stress tolerance in cotton by genetic engineering

Akmal M. Asrorov^{1,2*}, Mirzakamol S. Ayubov^{1*}, Mukhtor M. Darmanov¹, Sardor E. Narmatov¹, Akramjon Mamajanov¹, Ilhomjon E. Bozorov¹, Naim N. Khusenov¹, Ildiko Matusikova³, Zabardast T. Buriev¹ & Ibrokchim Y. Abdurakhmonov¹

¹Center of Genomics and Bioinformatics, Uzbekistan Academy of Sciences of, Tashkent, 111215, Uzbekistan

²Department of Natural Substances Chemistry, National University of Uzbekistan, Tashkent, 100174, Uzbekistan

³Department of Eco chemistry and Radioecology, University of Ss. Cyril and Methodius in Trnava, Trnava, 91701, Slovakia

*Email: mirzo.ayubov@gmail.com, akmal84a@gmail.com



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Abstract

Due to climate change, deserts are expanding, water reservoirs are drying, soil erosion is becoming more serious, and salinity areas are expanding worldwide. Among these disasters, soil salinization is one of the serious issues that affect agricultural production, with significant effects on plant development. Although plants have a solid adaptation to severe environmental conditions, their vulnerability to some abiotic stresses is still preserved in the plant genome. Cotton is a salt stress-tolerant crop among other main cash crops. However, its tolerance is limited in overwatered soil conditions or water-deficient soil. Several research investigations have been carried out to date to better understand salinity stress responses in various cotton species. The accumulation of salt due to irrigation-dependent practices exerts an adverse impact on crop productivity. However, this deleterious effect can be mitigated through a comprehensive understanding of the mechanisms by which certain plants flourish under saline conditions. Over the past few decades, there has been a notable augmentation in mechanistic comprehension, leading to the initiation of discovery-oriented methodologies aimed at discerning the genetic determinants of salt tolerance. Recent studies are showing the results of the manipulation of some important genes and proteins for salt tolerance using modern approaches. The identification of salt-resistance genes from salt-tolerant germplasm resources plays an essential role in improving the yield of cotton in saline soils. In this paper, we reviewed what has been achieved in cotton in terms of the development of its salt tolerance using genetic engineering.

Keywords

Defense-related genes; *Gossypium* species; Soil salinization; stress resistance

Introduction

The introduction of this paper provides a detailed overview of the critical aspects related to salinity. Salinity, characterized by elevated salt concentrations in soil or water, is a pivotal environmental factor with multifaceted implications for agricultural and ecological systems. This phenomenon stems from various sources, including both natural processes and significant human-induced factors, notably anthropogenic activities (1). To evaluate and monitor salinity levels accurately, various methods have been devised, encompassing the measurement of salt content in soil and water samples. One key unit for quantifying salinity is the electrical

conductivity, which reflects the ion concentration in a given medium (2). It is crucial to distinguish between salinity and sodicity, as they are distinct yet interconnected concepts, each with its unique impact on soil quality and plant growth. (3) Moreover, Survival in saline environments imposes a demand for elevated bioenergetic capabilities, as microflora are compelled to uphold osmotic equilibrium between their cytoplasm and the external environment. Microbial survival in saline conditions hinges upon the active exclusion of sodium ions from intracellular spaces, thus necessitating a substantial energy investment for optimal cellular function (4,5). Factors, including organic matter and cation exchange capacity content further modulate salinity levels, offering avenues for either increasing or decreasing the salt load in the soil, thus underscoring their significance in soil management and sustainability practices.

Due to poor agricultural practices and climate change, soil salinization has been one of the main abiotic limiting factors for plant and yield improvement. More than half of arable land in the World is expected to be salinized by 2050 (6). Many recent studies have demonstrated a significant drop in agricultural yields due to salt stress, raising worries about food security (7,8). As salinity stress has an increasing impact on cotton production, the implications are resonating in the global market. Recent studies on cotton provide a foundation to examine the intricate connections between these factors and offer insights into potential interventions to address the issues at hand.

Cotton plants that can tolerate high salt levels undergo several adjustments to adapt to salinity. These adjustments involve changes in their structure, functions, and biochemistry. They include increasing the proportion of roots to the aboveground canopy, enhancing the amount of chlorophyll, and altering the leaf structure. These adaptations primarily serve to prevent the harmful effects of excessive ions in the leaves, while also preserving water and safeguarding the photosynthesis process salinity stress has several negative consequences on the biochemical, physiological, and molecular properties of plants, as well as a reduction in output (9,10). The lands, prone to salinity, are continuously increasing worldwide (11). The salinity expansion causes many problems for both plants and the soil microbiome. Soil salinity is commonly known in more than 100 countries and ~20% of agricultural land is considered saline (12).

High salt levels in the soil hinder plant growth and development through various mechanisms. These include water stress, which is caused by reduced water availability due to the high salt concentration, as well as cytotoxicity resulting from the excessive uptake of ions like sodium (Na^+) and chloride (Cl^-). Additionally, salinity can disrupt the balance of essential nutrients required for plant growth (13). Sodium chloride (NaCl) is a widespread salt that inhibits plant germination and growth (14) and reduces plant height, fresh weight, leaf area, and dry weight. In addition, increases in leaf chlorophyll content do not affect leaf fluorescence (15). It is also known to

affect the evapotranspiration rate in plants and these changes lead to decreases in dry and fresh biomass (12). Besides, soil porosity leads to disruption of soil water potential (16).

Different strategies have been developed to overcome soil salt stress so far. Several papers, devoted to reviewing and summarizing scientific data on combatting salt stress in cotton, analyzed the roles of different genes and metabolites in *Gossypium* species. In line with developing genetically modified stress-tolerant plants, plant treatment with exogenous bioactive chemicals plays a significant role. These two strategies deserve more attention to overcome stressful conditions in plants (17). However, developing salt-tolerant plants using the methods of genetic engineering is the first step that can initially control various features responsible for growth and development along with plant tolerance to salt stress. In this paper, we reviewed cotton salt stress-related data that used genetic engineering and highlighted the main points around the alleviation of reactive oxygen species (ROS), providing osmotic pressure and ion exchange.

Genetic Engineering

Developing salt-tolerant plant varieties is one of the economically effective approaches to struggle against salt stress (18). Washing soil salt that helps to decrease its effect on plants can be helpful in some areas, but due to water scarcity, its effect is strongly limited (19). Thus, combatting salt stress requires breeding plant cultivars and varieties, resistant to salinity; the genes of these resistant cultivars can be applied to improve salt stress tolerance in cotton plants. Halophytes are generally used to find genes and can be efficiently used to develop salinity-resistant plant cultivars (20). Overexpression of genes, related to salinity and drought, is expected one of the few efficient strategies to struggle against salt stress and overcome its impact (21). These genes play pivotal roles in developing plant genotypes that tolerate abiotic stress including salt and drought stress (22). Late embryogenesis abundant (LEA) proteins, a large group that includes hydrophilic proteins, are of high interest due to their involvement in drought stress. In total, 136, 142, and 242 genes were identified in *G. arboreum*, *G. raimondii*, and *G. hirsutum*, in drought tolerance, respectively. LEA genes in *G. hirsutum* had genes with fewer introns, distributed in all chromosomes (23). Earlier, in cotton LEA transcripts were found to respond to salt and drought stresses (24).

The overexpression of bZIP transcription factor (*GhABS2*), a regulator of abscisic acid-mediated abiotic signaling pathways in cotton, enhanced drought and salt tolerance by the regulation of genes associated with salt stress responses and abscisic acid. Enhanced levels of proline and activities of catalase and superoxide dismutase were in correspondence with other results (25).

Scientists used a variety of approaches to solving salt stress problems in less than a century. Because the genetic bottleneck effect limits the availability of salt tolerance cotton varieties, breeders must identify important genes/loci and transfer them to local high-

yielding cotton cultivars using conventional or modern methods. In summary, while conventional breeding has historically been the primary method for developing salt-tolerant cotton varieties, modern breeding techniques offer significant advantages in terms of precision, speed, and access to genetic diversity. Contemporary techniques such as genetic engineering and gene editing hold the promise of expediting the advancement of cotton cultivars endowed with heightened resilience to salt-induced stress. However, it is essential to consider regulatory and environmental factors when employing these modern approaches. The integration of different approaches such as modern breeding and conventional breeding with genome editing and genetic analysis may allow the development of salt-tolerant cotton plants against the intensified effects of the changing environment (Fig. 1.) (26).

The expression of genes from other plant species

Dehydration-responsive element binding (DREB) transcription factors are means contributing to the regulation of stress tolerance in cotton (27). The overexpression of potato DREB2 in cotton led to higher expressions of antioxidative enzymes such as peroxidase, superoxide dismutase, glutathione S-transferase, and stress-tolerant genes. Thus, the overexpression enhanced cotton plant drought tolerance by augmenting the capacities of ROS scavenging and antioxidative enzymes (28). Synergistic effects of the co-expression of rice SUMO E3 ligase gene (*OsSIZ1*) and Arabidopsis thaliana vacuolar pyrophosphatase gene (*AVP1*) in cotton plants increased the plant crop production by 133 percent and 81 percent in two field experiments. The synergistic effects of *AVP1/OsSIZ1* genes in cotton were concluded as a viable strategy

for developing abiotic stress-tolerant crops to grow in arid and semi-arid regions (29). *MAPK* cascade signaling is another mechanism, used by cotton plants to protect against salt stress. Thus, molecules of this cascade can be used as indicators to prognose the salt stress resistance of plants (30). *MAPK* gene isolated from *G. hirsutum*, termed *GhMPK17*, was found upregulated in *A. thaliana* when grown under NaCl, mannitol, and abscisic acid. The gene expression in *A. thaliana* enhanced seed germination and root elongation under abiotic stress conditions (31).

Using genome-wide analysis and RNA sequencing, 33 single-nucleotide polymorphisms in *G. hirsutum* were identified. Besides, the authors identified 35 genes that were involved in salt stress responses. By qRT-PCR, 12 genes were verified (32). Genome-wide analysis of four cotton species *G. arboreum*, *G. raimondii*, *G. hirsutum*, and *G. barbadense* enabled the identification of Tubby-like proteins-expressing TLP genes involved in salt stress. qRT-PCR analysis validated the high sensitivity of *GhTLP11A* and *GhTLP12A.1* genes to drought and salt stress. Other network elements confirmed the functional significance of these genes in these stress responses (33).

Gh3 genes, involved in homeostasis due to their ability to conjugate auxin and bind IAA to amino acids, are expected one of the key points in salt tolerance. By RNA expression analysis in *G. hirsutum*, 10 genes were identified as salt-stress responsive. Their silencing in *G. arboreum*, *G. raimondii*, and *G. hirsutum* caused a significant reduction in plant tolerance to salt and drought stress. The gene silencing significantly reduced the levels of chlorophyll, relative leaf water content, and superoxide dismutase activity (34). The transcriptomic analysis to defer ROS gene evolution under salt stress was carried out

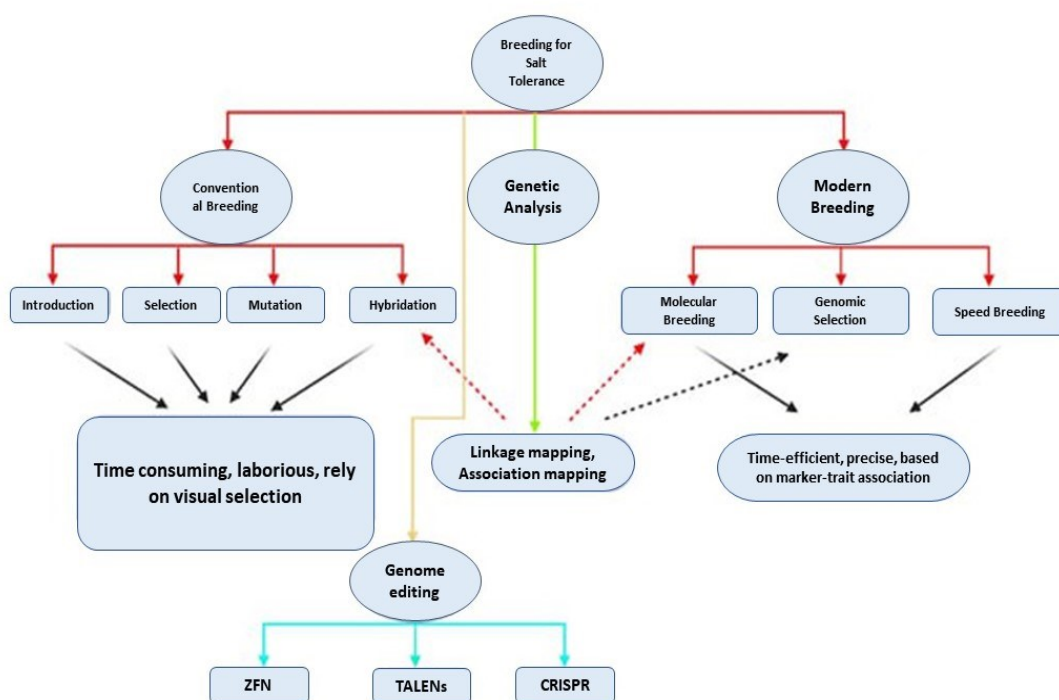


Figure 1. Using genetic engineering in combination with other approaches to develop salt-tolerant cotton genotypes with the expression of salt-resistant genes

in cotton. The authors identified 260, 261, and 515 genes, related to the ROS network, in *G. arboreum*, *G. raimondii*, and *G. hirsutum*, respectively. *CSD1* and *RBOH6*, playing significant roles in fiber development, were involved in cotton plant salt stress as well (35). A group researcher identified 672, 379, and 374 proteins in *G. hirsutum*, *G. arboreum*, and *G. raimondii*, encoded by CYPs genes. A common feature of stress-related genes was that the protein, encoded by them, were hydrophilic. Gene-silenced plants revealed a lower level of ascorbate peroxidase, proline, and glutathione under salt and drought stress. Silencing of *CYP450* caused a high level of oxidative injury (36). The salt overly sensitive 1 (*SOS1*) gene is one of the salt stress-responsive genes playing a pivotal role in plant salt stress. Encoding plasma membrane Na⁺/H⁺ antiporter regulates plasma Na⁺ under salt stress. Cotton plants expressing GhSOS1 and transformed with virus-induced gene silencing revealed increased salt sensitivity. Besides, plants demonstrated lowered growth and increased Na⁺ content in leaves, stems, and roots (37). Studying the responses of small RNA in cotton to salt shock and long-term salt stress revealed 88 and 75 differentially expressed genes. The *miR390/tasiRNA-ARFs/ARF4* pathway was discovered to be altered in salt-stressed plants (38). Histone acetyltransferases (*HATs*) are a group of genes that play significant roles in chromatin regulation. Among them, the expression level of several genes including *GhHAC-1501*, *-1502*, *-1503*, *GhHAG1501*, *-1504*, and *GhHAF-1501* enhanced in response to salt stress. *HATs* were differentially controlled in response to salt stress, cold, drought stress, and heavy metals in cotton plants. In general, these genes were concluded to be associated with responses to various abiotic stresses (39). Genome-wide analysis of callose synthases (*CalSs*) allowed the identification of these gene family members in cotton plants. Based on the sequences of *CalSs* from *A. thaliana*, 27 and 28 genes were found in *G. hirsutum* and *G. barbadense*, respectively. Transcriptome analysis and qRT-PCR analysis under abiotic stresses including salt, cold, heat, and drought enabled the prediction of their roles in fiber development and salt response (40).

Conclusion

This review has underscored a major avenue for combating salt stress in cotton: genetic engineering. Genetic engineering in cotton has shown promise in enhancing plant salt tolerance. The exploration of various genes and transcription factors, such as *DREB2*, *GhABS2*, and *MAPK* signaling cascade, has provided valuable insights into developing salt-resistant cotton cultivars. The identification of specific genes and proteins, like LEA proteins, *TLP* genes, *Gh3* genes, and ROS-related genes, has offered potential targets for genetic manipulation to bolster salt stress tolerance. As technology advances, modern breeding methods, including gene editing and genetic engineering, offer precise and efficient means of developing salt-tolerant cotton varieties. However, it is essential to navigate regulatory and environmental considerations when deploying these cutting-edge techniques.

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Authors contributions

AA, MA – coordinated, wrote, and revised manuscript; MD, SE, AM, IB, NK – collected and analyzed literature, drafted manuscript; IM, ZB, IA – critically read and edited manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Table 1. The important genes, used to obtain salt tolerance cotton lines and varieties.

Sl.No.	Name of the foreign gene	Name of the source plant	Salt stress-resistant actions upon engineering in cotton	Reference
1.	<i>StDREB2</i>	<i>Solanum tuberosum</i> L.	Overexpression	(28)
2.	<i>AVP1</i>	<i>A. thaliana</i>	Overexpression	(29)
3.	<i>OsSIZ1</i>	<i>Oryza sativa</i>	Overexpression	(29)
4.	<i>GaJAZ1</i>	<i>G. arboreum</i>	Overexpression	(21)
5.	<i>GhMPK17</i>	<i>G. hirsutum</i> L.	Overexpression	(31)
6.	<i>Gh_D07G1197</i> , <i>Gh_A13G2057</i>	<i>G. hirsutum</i> L.	Gene silencing	(36)
7.	<i>GhSOS1</i>	<i>G. hirsutum</i> L.	Gene silencing	(37)

Ethical issues: None.

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