

Role of molecular breeding in understanding salinity tolerance in cotton: limitations and perspectives

Shicong WANG^{1,2}, Meiling HOU¹, Hadiqa HASSAN³,
Fatima M. ABBAS⁴, Zehbah A. AL AHMAD⁵, Sameer H. QARI⁶,
Nurul A.B. HANIFAH^{2*}

¹Baicheng Normal University, College of Life Sciences, Baicheng 137000, Jilin, China; wushicong@qq.com (S.W.);
meilingnm@163.com (M.H.)

²City University Malaysia, City Graduate School, Kuala Lumpur 50470, Malaysia;
nurul.azlen@city.edu.my (N.A.B.H.) (*corresponding author)

³University of Sialkot, Department of Biotechnology, Sialkot, Pakistan; hadiqahassan7@gmail.com (H.H.)

⁴King Khalid University, Faculty of Sciences and Arts, Department of Biology, Dabran Al-Janoub 61421,
Saudi Arabia; fmelamin@kku.edu.sa (F.M.A.)

⁵King Khalid University, College of Science, Department of Chemistry, Abha 61413, Saudi Arabia; Zalsbwal@kku.edu.sa (Z.A.A.A.)

⁶Umm Al-Qura University, Al-Jumum University College, Department of Biology, Makkah 21955,
Saudi Arabia; shqari@uqu.edu.sa (S.H.Q.)

Abstract

Cotton is a significant fiber crop globally and has a considerable share in many countries' gross domestic product (GDP). It is the most critical contributor to the textile industry and provides income to millions of farmers. Salt stress is abiotic stress, decreasing the cotton yield on a large area. Salt stress is a polygenic trait, and cotton's response to salt stress involves a complex gene pathway. Breeders have been breeding novel salt-tolerant cotton genotypes for decades to sustain their growth on salt-affected soils. In recent years, cotton breeders have employed several breeding tools like hybridization, backcrossing, and mass selection to develop tolerant genotypes. Still, due to several limitations, these techniques are being replaced by novel molecular breeding tools. With the advancement in molecular breeding, the speed to improve crop tolerance to salt stress has been increased. Quantitative trait loci (QTL) mapping, genome-wide association studies (GWAS), transcription factors (TFs) analysis, and transcriptome have identified several genomic regions for salinity tolerance in cotton. At the same time, genetic engineering and clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) led to the development of salt-tolerant cotton cultivars. Genetic engineering is key in breeding transgenic cotton cultivars resistant to multiple abiotic stresses. CRISPR/Cas9, a new gene manipulation technique, is used to edit the genes for salt tolerance in cotton without any biological barrier. CRISPR/Cas9 could be a more powerful tool to manipulate the desired cotton genome against salinity tolerance. These research and breeding tools have been successfully used in genetic research and breeding for drought tolerance in cotton. This unique review presents a full overview of the use of different molecular tools and their role in enhancing salt tolerance in cotton. Using this information, cotton breeders can understand the salinity tolerance mechanism in cotton by choosing the most reliable genetic breeding tool.

Received: 27 Nov 2024. Received in revised form: 15 Feb 2025. Accepted: 00 Xxx 2025. Published online: 00 Xxx 2025.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Keywords: abiotic stress; cotton genome; genes; molecular breeding; QTL

Introduction

The beginning of the 21st century is noticeable by a deterioration in natural resources, environmental pollution, soil and water reservoir salinization, and severely threatening health (Ju *et al.*, 2021; Shahbaz and Ashraf, 2013). Soil salinity causes a noteworthy decline in crop yield (An *et al.*, 2025; Guo *et al.*, 2023; Phour & Sindhu, 2023; Wei *et al.*, 2017; Yu *et al.*, 2020; Zeb *et al.*, 2016; Zhou *et al.*, 2023) poses a risk to crop sustainability, production, eventually global food security. Food and Agricultural Organization (FAO, 2011) reported that around 45 million hectares of irrigated land are disturbed by soil salinity, while predictions revealed that more world arable lands will be disturbed in the future (Jamil *et al.*, 2011). Salt-affected areas are increasing quickly, reducing agricultural productivity (An *et al.*, 2025; Farooq *et al.*, 2022; Ghassemi-Golezani *et al.*, 2024). Salinity stress arises in soil by the accumulation of sulfates and chlorides of calcium, magnesium, and sodium. Excessive accumulation of Ca^+ , Na^+ , and K^+ leads to salt toxicity in soils (Murtaza *et al.*, 2025; Wu *et al.*, 2023b). A high concentration of Na competes with K^+ ions, disturbs K^+ homeostasis, and triggers K^+ leakage, which leads to K^+ deficiency in plants (Nieves-Cordones *et al.*, 2016). Cotton is one of the most economically significant crops which is used in the textile industry to make a large number of products, and at the same time, it is a substantial source of farmer's income in many regions (Maryum *et al.*, 2022; Muhammad *et al.*, 2025; Sun *et al.*, 2023), cultivated in arid as well as semi-arid areas where salt and drought are a common threat to cotton growth and production. The genera *Gossypium* comprises 50 species, a rich source of genes for yield, fiber quality, and resistance to environmental stresses (Saddique *et al.*, 2022). Salt stress leads to significant changes in the morphology, physiology, and biochemistry of crops (Abdelraheem *et al.*, 2018; Anwar *et al.*, 2024; Chaudhary *et al.*, 2024; Rasheed *et al.*, 2022; Zafar *et al.*, 2025), resulting in reduced growth and production (Abdelraheem *et al.*, 2020a) (Figure 1). By viewing climate changes, it has become imperative to increase the cotton yield by up to 40% (Abdelraheem *et al.*, 2021a).

Using molecular techniques like QTL mapping is one of the most significant ways to recognize the potent genomic areas controlling salinity tolerance in cotton. Hundreds of QTLs with substantial contributions to salinity stress have been identified. QTL identification can help to develop salt-tolerant cotton varieties. Association mapping using linkage disequilibrium is one of the most powerful ways to identify the regions linked to particular variants of phenotypic traits (Saeed *et al.*, 2014). Association mapping can dissect a large number of alleles as compared to linkage mapping. Unfortunately, studies on the identification of salt-tolerant QTL/genes by association mapping (Du *et al.*, 2016) and linkage mapping are limited (Diouf *et al.*, 2017). Yuan *et al.* (2019) identified 33 SNPs and 13 QTLs for salinity tolerance in cotton using association mapping. They concluded that these findings could increase our understanding of molecular regulatory pathways of salinity tolerance in cotton (Yuan *et al.*, 2019).

TFs and transcriptome analysis identify the protein families and genes regulating salinity stress tolerance in cotton (Li *et al.*, 2025; Liu *et al.*, 2025; Peng *et al.*, 2025). Several TFs and transcriptomes have been identified as involved in salinity tolerance in cotton. TFs reveal the distant pathways associated with salinity tolerance in cotton. An earlier study recognized 2356 differently expressed genes in cotton after treatment with salt stress, of which 94 were TFs. These TFs /genes are significant genetic reservoirs that enhance cotton genotypes' salt tolerance (Long *et al.*, 2019). CRISPR/Cas9 and genetic engineering are the most potent tools to breed salt-tolerant cultivars. Many studies revealed the role of transgenic cotton cultivars in combating salinity stress. The breeding of transgenic cotton with improved tolerance to salt stress has great agronomic value (Huijun *et al.*, 2010). CRISPR/Cas9 is a new gene editing technique that addresses environmental challenges by editing the gene of interest without biological barriers. The use of CRISPR/Cas9 to edit the salt-

tolerant genes is limited and requires further studies to expand its role in future studies. We used Google, Google Scholar, and PubMed to search for relevant literature and write the manuscript. Many published papers deal with the mechanism of salinity tolerance in cotton; however, a detailed overview of this aspect is rarely reported or missing. Hence, this is the first detailed review focusing on molecular breeding tools to enhance salinity tolerance in cotton. This review aims to present an updated picture of improving salt tolerance in cotton using molecular breeding methods to give concise information to cotton breeders. This information can help cotton breeders speed up salt-tolerant breeding.

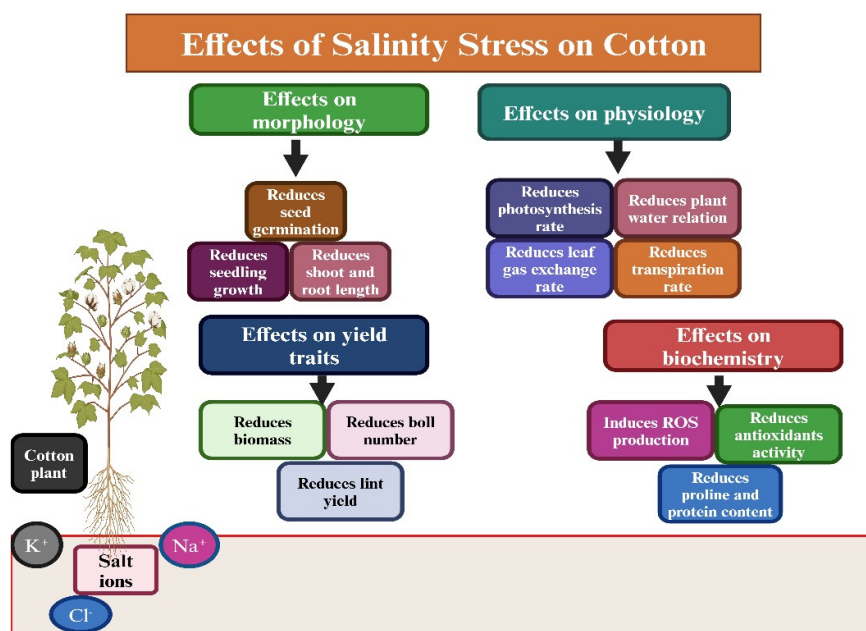


Figure 1. Salinity stress influences growth, yield, and fiber quality. Salinity stress also affects the uptake of nutrients and stops plant reproduction. Salinity stress also reduces plant water relations, leaf gas exchange, and transpiration rates. Salinity stress reduces lipid content, protein content, and activities of plant growth hormones. This figure was made with bioRender.com

Effects of salinity stress in cotton

Soluble salts induce ionic and osmotic imbalance (Shehzad *et al.*, 2019a). Salinity stress reduces plant growth through three mechanisms (Guo *et al.*, 2023). A high salt concentration of 360 mmol L^{-1} retarded seed germination and seedling growth (Yan *et al.*, 2019). Salinity stress reduces plant height and photosynthesis (Zafar *et al.*, 2022). Salt stress reduced the relative water absorption and water absorption rate in cotton genotypes (Wu *et al.*, 2023c). Salinity stress reduced the osmotic potential of cotton when exposed to NaCl levels (Meloni *et al.*, 2001). Results exhibited that nitrogen (N) and potassium (K) uptake were significantly decreased by salinity stress (Chen *et al.*, 2010). Another study showed that salinity stress (200 mol m^{-3}) reduced the concentration of Ca^{2+} and Mg^{2+} in leaves (Meloni *et al.*, 2001). These mechanisms include an imbalance in nutrient ions that decreases NO_3^- , PO_4^{3-} , and K^+ uptake with high NaCl content and osmotic stress interrupting water accessibility (Shehzad *et al.*, 2019a). Salinity stress negatively affects cotton growth and production (Zafar *et al.*, 2022). Salinity stress (150 mM) reduced the rate of photosynthesis, activities of sucrose phosphate synthase (SPS), and RuBP carboxylase in cotton cultivars (Desingh and Kanagaraj, 2007).

Salinity stress also suppresses the functions of enzymes linked with a metabolic pathway (Sharif *et al.*, 2019). Ju *et al.* (2021) evaluated the cotton cultivars to investigate the effects of salinity stress on different traits. Salinity stress (10.59~11.08 dS m⁻¹) decreased the concentration of soluble sugar, starch, and sucrose (Ju *et al.*, 2021). Another study showed that salinity stress reduced the protein content in cotton (Meloni *et al.*, 2001). Reactive oxygen species (ROS) altered cellular metabolism *via* damaging DNA and proteins (Doğan *et al.*, 2012). Salt stress reduced bolls and lint yield in cotton plants, as studied by Ibrahim *et al.* (2019). Salt stress reduces the dry biomass of cotton cultivars by reducing growth, seed cotton yield per plant⁻¹ and other traits (Sikder *et al.*, 2022).

Genetic mechanism of salinity tolerance in cotton

Investigating cotton response to salinity stress, tolerance mechanisms, and salinity management methods may contribute to formulating the techniques to increase cotton growth under salinity stress (Liu *et al.*, 2025; Sharif *et al.*, 2019). At the molecular level, plants activate several genes, enzymes, and proteins to counter the harmful effects of salt stress. Studies showed several genes involved in cotton response to salinity tolerance (Fang *et al.*, 2025). Earlier studies showed that the rice gene *SNAC1* enhanced salt tolerance in cotton (Liu *et al.*, 2014b). The involvement of ion transporters in salt stress response has been studied. The Na⁺/H⁺ antiporter plays a key role in salt stress, and overexpression of *AtNHX1* improved salt tolerance in cotton (He *et al.*, 2005; Lv *et al.*, 2008). The ROS scavengers *GhCAT1* and *GhMT3a* also participate in salt tolerance in cotton (Luo *et al.*, 2013; Xue *et al.*, 2009).

Improvement of the antioxidant defense system reduces the risks of oxidation-related damage. Salinity tolerance is a polygenic trait governed by numerous genes and their network (Diouf *et al.*, 2017). Salinity tolerance meaningfully differs among cotton germplasm (Yuan *et al.*, 2019). Screening of salt-tolerant germplasm is imperative to breed the salt-tolerant genotypes of cotton and recognize the molecular mechanisms linked with salt tolerance. Breeding tolerant varieties is the most promising way to counter soil salinity (Yuan *et al.*, 2019). The effects of salinity stress on nucleic acids, protein synthesis, and plant-water relation should be deeply investigated to establish a strong counter plan to decrease the danger of salt stress on crops.

QTL mapping analysis of salinity tolerance in cotton

QTL mapping is one of the most powerful tools to identify the potential genomic regions contributing to abiotic stress tolerance in crops (Du *et al.*, 2016; Li *et al.*, 2024; Shakir *et al.*, 2025). QTL mapping facilitates the identification of salt-tolerant QTL in cotton. It is a powerful way to recognize the molecular mechanism of salinity tolerance in cotton. QTL analysis helps to understand whether the particular trait is affected by a few loci with major effects or many loci with small effects (Guo *et al.*, 2024). Marker-assisted selection (MAS) is a powerful way to exploit several QTLs at once. After identifying QTL with a positive effect on traits of interest, the next step is to combine them along the same line. This process is called QTL pyramiding (Huang & Zhao, 2020). QTL pyramiding has been successfully used in cotton to improve salinity tolerance, as reviewed by Shehzad *et al.* (2019b). An F_{2:3} mapping population was assessed to identify the QTL linked with salt tolerance. Eleven consistent QTLs were detected on eight chromosomes, including 9, 11, 15, 16, 21, 23, 24, and 26. *qRL-16-1* was a major salt-tolerant QTL, explaining significant phenotypic variance in two environments. This QTL shows the significance of root length (RL) in salinity tolerance in cotton. More studies are required to evaluate the role of other QTLs identified in this study (Oluoch *et al.*, 2016). Diouf *et al.* (2017) identified 66 QTL for 10 salinity tolerance traits in three environments. Out of 66 QTLs, only 14

were consistent across the environments. Resistant parent (CCRI35) contributed four QTLs, which could improve salinity tolerance in the population. Fourteen consistent QTLs identified in this study should be studied for their possible use in molecular breeding (Diouf *et al.*, 2017). QTL identification at the germination stage is one of the critical objectives of the cotton breeder. QTL identification at germination can help avoid the toxic effects of salt stress in the adult stage. A stable salt-tolerant QTL, QTL *qSalt-A04-1*, was identified for relative germination rate (RGR) after salt treatment. This novel QTL significantly contributed to salinity tolerance at the germination stage. Besides this, this novel QTL harbored candidate genes, a potential genetic resource for salt-tolerant breeding. Hence, QTL for RGR can be targeted to accelerate the marker-assisted selection in cotton (Guo *et al.*, 2021). Guo *et al.* (2022b) evaluated 177 RIL population and their parents under salt stress conditions. Germinal potential (GP) and germinal length (GL) were studied under salt stress conditions. QTL, *qGR-Chr4-3*, *qFER-Chr12-3*, and *qFER-Chr15-1* (Table 1) representing field emergence rate (FER) were detected as salt-tolerant QTL. Additional studies are required for further functional characterization of these QTLs and their use in molecular breeding (Guo *et al.*, 2022b).

In another study, 55 QTL were detected for salt tolerance in the RIL population. RIL population is homogenous or homozygous and considered valid for QTL mapping. This population can be maintained for a long time and used repeatedly. The QTL, *qPH5* was commonly mapped under salt and drought stress and considered stable QTL for abiotic stress tolerance. *qFSW6* was identified for fresh shoot weight (FSW) under salt stress conditions (Abdelraheem *et al.*, 2018). Identifying QTL for fiber quality and length under salt stress conditions should be a key target of plant breeding. Three QTLs (*qFL-Chr1-1*, *qFL-Chr5-5*, *qFL-Chr24-4*) for fiber length were identified under salt stress and control conditions. These QTLs can facilitate the development of salt-tolerant cotton cultivars with improved fiber length (Guo *et al.*, 2021). A recent study by Su *et al.* (2021) identified the QTL controlling yield, seedling, and fiber quality traits under salt stress in cotton. Three QTL *qFL-ChrD11-3*, *qFM-ChrA13-2*, and *qFM-ChrD05-1* were stable and spotted for fiber quality characters under several situations in two years. These QTLs can be exploited *via* molecular breeding to improve the fiber quality under salt stress in cotton (Su *et al.*, 2021). In another study, 161 RIL were evaluated under salinity stress conditions. One QTL, *qDSW-12*, for dry shoot weight (DSW) was commonly mapped under salt and drought stress. Hence, DSW can be improved under salt stress in cotton, as proven by this study (Abdelraheem *et al.*, 2020b).

QTL identification for morphological traits under salt stress in cotton is essential to improving salt tolerance in field conditions. Three QTLs for PH, one for leaf number (LN), one for fresh root weight, and one for root fry weight (RDW) were detected under salt stress (200 mM NaCl) by Tiwari (2012). These QTLs can facilitate the marker-assisted selection for salt tolerance in cotton (Tiwari, 2012). Han *et al.* (2022) identified 30,089 eQTL associated with cotton growth and salt tolerance and concluded that these eQTL could be used to develop improved cotton cultivars. Hence, more studies are required to unfold the potential role of these QTLs in salinity tolerance in cotton (Han *et al.*, 2022). These studies revealed the role of novel genomic regions in enhancing salt tolerance in cotton. QTL mapping studies on cotton boll weight and other physiological traits under salt stress are limited. QTL mapping studies for physiological characteristics would boost the development of physiological-based salt tolerance in cotton. This research gap must be covered in future studies.

Table 1. QTL for salinity tolerance in cotton

Population	QTL	Chromosome	References
177 RILs	<i>qLP-Chr5-3</i>	5	(Guo <i>et al.</i> , 2024)
177 RIL	<i>qGR-Chr4-3, qFER-Chr12-3, qFER-Chr15-1</i>	4, 12, 15	(Guo <i>et al.</i> , 2022b)
588 F ₇ RIL	<i>qSalt-A04-1</i>	4	(Gu <i>et al.</i> , 2021)
RIL	<i>qFL-ChrD11-3, qFM-ChrA13-2, qFM-ChrD05-1</i>	11, 13, 5	(Su <i>et al.</i> , 2021)
RIL	<i>qFL-Chr1-1, qFL-Chr5-5, qFL-Chr24-4</i>	1, 5, 24	(Guo <i>et al.</i> , 2021)
RIL	<i>qDSW12</i>	12	(Abdelraheem <i>et al.</i> , 2020b)
97 F _{5:9} RILs	<i>qFSW6, qPH5</i>	6, 5	(Abdelraheem <i>et al.</i> , 2018)
277 F _{2,3} populations	<i>qFW_A06_150.1</i>	AO6	(Diouf <i>et al.</i> , 2017)
F _{2,3} population	<i>qRL-16-1</i>	16	(Oluoch <i>et al.</i> , 2016)

RIL: recombinant inbred lines, DSW: dry shoot weight, FSW: fresh shoot weight, GR: germinal ratio, Chr: chromosomes, RL: root length, RGR: relative germination rate.

Genome-wide association study (GWAS) analysis for salt tolerance in cotton

GWAS is the most significant and potent technique to determine the trait marker association and to identify the genes/QTL for salinity tolerance in cotton (Wu *et al.*, 2023a). A detailed and comprehensive analysis of GWAS-based identified genes/QTL is critical to understanding the molecular basis of salinity tolerance in cotton. A recent study developed a multi-parent advanced generation intercross population of 550 RIL to identify the QTL for salt tolerance at the seedling stage. A total of 23 QTLs were detected for salt tolerance. These QTLs were detected for plant height and dry shoot weight (DSW). A common QTL, *qPH-1*, was detected under drought and salt stress (Abdelraheem *et al.*, 2021b). Previously, many studies focused on QTL identification at the seedling stage. Yuan *et al.* (2019) employed GWAS and RNA sequencing to detect the QTL in 196 upland cotton genotypes. For salt tolerance, 33 SNPs, 13 QTL, and 98 candidate genes were identified. These findings can improve our understanding of molecular regulatory mechanisms of cotton tolerance to salt stress (Yuan *et al.*, 2019). Sun *et al.* (2018) also employed GWAS to study the association of marker traits. They detected 23 SNPs linked with two salt-tolerant traits, relative survival rate (RSR) and salt tolerance level (STL). The two SNPs (i46598Gh and i47388Gh) on D09 were simultaneously related to the two characters (Sun *et al.*, 2018).

Cotton tolerance to salinity stress is not well understood. In another association analysis, 419 cotton accessions were used to identify the SNPs related to salt tolerance traits. A total of 17,264 SNPs were found for tolerance-related features. Twenty key genes around SNPs A10_95330133, and D10_61258588, linked with leaf relative water content and fresh weight, were selected. These results provide valuable information about salt tolerance at the seedling stage (Yasir *et al.*, 2019). Xu *et al.* (2021) performed GWAS for salt tolerance at the seedling stage in upland cotton genotypes. A total of 51,060 SNPs were identified on 26 chromosomes. The association between chromosomes A13 and Do8 for relative plant height (RPH), Ao7 for relative shoot fresh weight (RSFW), and Ao8 and A13 for relative shoot dry matter weight (RSDW) were expressed in both environments. These associations were likely the stable QTL (Xu *et al.*, 2021). GWAS identified nine SNPs

linked with salt tolerance traits, such as relative stem length (RSL), relative fresh weight (RFW), and relative water content (RWC) in Asiatic cotton. Forty candidate genes were associated with salt tolerance (Dilnur *et al.*, 2019).

The latest study identified 27 SNP markers closely associated with salt tolerance traits and 15 SNP markers correlated to the salt tolerance index. This study provides a theoretical basis for the genetic improvement of upland cotton (Zheng *et al.*, 2021b). Another GWAS revealed that 31, 19, and 15 simple sequence repeats (SSR) markers were linked with relative germination rates of seeds (RGR) under salt stress. Four candidate genes (*GhPIP3A*, *GhSAG29*, *GhTZF4*, and *GhTZF4a*) (Table 2) exhibited differential expression in salt-tolerant and sensitive genotypes (Sun *et al.*, 2019). Some GWAS analyses were conducted for components of lint yield, lint percentage (LP), and single ball weight (SBW) (Zhu *et al.*, 2020). A major GWAS analysis was conducted using 316 *Gossypium hirsutum* accessions. Based on GWAS analysis, 91 and 42 QTL were correspondingly recognized for LP and SBW. 10 genes were detected from 8 stable QTL of LP, and these genes were expressed during fiber development (Zhu *et al.*, 2020).

A GWAS analysis identified that SSR is linked to salinity tolerance in cotton. Saeed *et al.* (2014) evaluated a set of 109 cotton genotypes under salt stress (Saeed *et al.*, 2014). They have detected several SSR makers (BNL3103 (D6), NAU478 (D8), and BNL3140 (D9)), which were significantly associated with salinity tolerance. A statistical tool, STRUCTURE software, recognized five sub-populations in this cotton germplasm. These populations and markers can be used in the molecular breeding of cotton to develop salt-tolerant cultivars (Saeed *et al.*, 2014). Another association study was conducted to identify the markers associated with salt tolerance in cotton. 106 microsatellite markers were used for 323 cotton germplasms exposed to salinity stress.

Significant associations between salt tolerance and markers suggested that association mapping could complement and increase QTL information for the MAS program. This study also used STRUCTURE software to detect polymorphism (Jia *et al.*, 2014). Zhao *et al.* (2016) revealed that identifying SSR markers contributed to marker-assisted selection (MAS), which can enhance the efficiency of traditional breeding. Association mapping was used to determine the markers associated with salt tolerance in cotton cultivars. A total of 148 loci were detected in 74 SSR markers. Finally, eight SSR markers contributed to salt tolerance in cotton (Zhao *et al.*, 2016). Although there are many detailed studies about salt tolerance in cotton, the molecular mechanism of salinity tolerance is still not fully understood. Future studies should focus on identifying QTL linked with biochemical traits under salinity stress. Future studies would open new dimensions of the molecular basis of salt tolerance in cotton.

Table 2. GWAS-based mapped QTL/genes for salt tolerance in cotton

Population	Genes/QTL/SNP	Traits	References
Cotton genome	33 <i>C5-MTases</i>	Salt tolerance traits	(Yang <i>et al.</i> , 2024a)
214 accessions	30,089 eQTLs in 10,485 genes	Salt tolerance traits	(Han <i>et al.</i> , 2022)
MAGIC (550 RIL)	23 QTL, <i>qPH-1</i>	Plant height (PH)	(Abdelraheem <i>et al.</i> , 2021)
149 cotton plant materials	<i>Gb_A01G0908</i> , <i>Gb_D08 G1308</i>	Salt tolerance traits	(Zheng <i>et al.</i> , 2021)
215 accessions of Asiatic cotton	40 candidate genes	relative stem length (RSL), relative fresh weight (RFW)	(Dilnur <i>et al.</i> , 2019)
196 upland cotton genotypes	33 SNPs, 13 QTL, and 98 candidate genes	Salt tolerance traits	(Yuan <i>et al.</i> , 2019)
503 upland cotton accessions	<i>GbPIP3A</i> , <i>GbSAG29</i> , <i>GbTZF4</i> , and <i>GbTZF4a</i>	RGR	(Sun <i>et al.</i> , 2019)
713 upland cotton accessions	23 SNP, 280 genes	relative survival rate (RSR), and salt tolerance level (STL)	(Sun <i>et al.</i> , 2018)
134 samples of upland cotton cultivars	148 in 74 SSR	Salt tolerant traits	(Zhao <i>et al.</i> , 2016)
74 cotton cultivars	95 significant associations (SSR), 117 elite alleles	Germinative index, seedling stage, physiological index, and four seedling stage biochemical indexes	(Du <i>et al.</i> , 2016)
109 cotton variety	250 SSR markers	Salt tolerance traits	(Saeed <i>et al.</i> , 2014)
323 <i>Gossypium hirsutum</i> germplasms	106 microsatellite markers	Salt tolerance traits	(Jia <i>et al.</i> , 2014)

Transcription factors analysis of salinity tolerance in cotton

The plant response to salinity stress requires differential gene expression regulated by TFs. These TFs are key components in regulating transcription initiation rate to modulate the stress-associated genes for abiotic stress signaling cascades (Furihata *et al.*, 2006; Yamaguchi-Shinozaki and Shinozaki, 1994; Liang *et al.*, 2016). Different TFs like ethylene responsive factor (ERF), basic leucine zipper (bZIP, WRKY, and MYB) can regulate the expressed genes (Lan Thi Hoang *et al.*, 2017; Long *et al.*, 2019). Regulation of gene expression is responsible for synthesizing and degrading proteins, metabolites, and enzymes. These constitute plant defense against abiotic stresses (Barbosa *et al.*, 2013). These TFs are critical for plant breeders to develop tolerance against abiotic stresses (Liu *et al.*, 2014a). bZIP is one of the largest families of TFs, mainly involved in plant response to abiotic stresses. They are an essential regulator of ABA-mediated abiotic stress signaling pathways in crops (Liang *et al.*, 2016). A member of bZIP, *GhABF2*, was earlier cloned and characterized for salinity tolerance in cotton. Transcriptome analysis indicated that expression of *GhABF2* increased the salinity tolerance by enhancing the expression of genes related to ABA and salt response. On the other hand, this TF controls the major regulatory pathways linked with the activity of proline and antioxidant enzymes like superoxide dismutase and catalase (Liang *et al.*, 2016).

Recently, another member of bZIP TF, *GhABF3* (Table 3), was studied for its role in salinity tolerance in cotton. This gene regulates salt stress through various pathways and networks. Overexpression of this gene increased the cotton tolerance to salt stress, resulting in a reduced level of oxidation in the cell and increased transcriptional abundance of ABA-related genes (Zhang *et al.*, 2022). This TF also upregulated the expression of ABA-inducible genes under salt stress. These studies indicated the role of bZIP TF and its significance for molecular breeding (Zhang *et al.*, 2022). Different cotton genotypes have different levels of salt tolerance. A TCP TF, *GbTCP4*, is mainly known for its regulatory role in cotton growth and development under salt stress; however, its role in sea-land cotton is unknown. Under NaCl treatment, the cotton genotype showed increased tolerance due to enhanced expression of the *GbTCP4* gene. RNA-seq analysis exhibited that *GbTCP4* influenced the transcription levels of genes across numerous abiotic stress-related metabolic pathways and networks. Furthermore, *GbTCP4* triggered the transcription of *GbUVR8* and *GbbHLH130* by binding to the gene promoter. *GbTCP4* induced the expression of stress-related genes in cotton and regulated salt responses ABA-dependently (Wang *et al.*, 2022b).

A detailed and critical analysis of different members of TF families is essential to strengthening the molecular breeding program in cotton. Studies showed that C₂H₂ zinc finger proteins, C₂H₂-ZFP, were involved in cotton response to salt stress. Rehman *et al.* (2021b) obtained 47, 45, 94, and 88 genes from diploid A2, D5, AD1, and AD2 cotton. Data integration showed that *GhZAT34* and *GhZAT79* might regulate NaCl tolerance in cotton by enhancing seed germination rate (Rehman *et al.*, 2021b). KNOX TFs are known for their crucial role in seed development and response to salt stress in cotton. The divergence and evolutionary history of the KNOX gene family have been studied in cotton. Silencing of *GhKNOX2* increased salt tolerance in cotton at the seedling stage by regulating the genes network linked to flowering time and plant development stage (Zhang *et al.*, 2021c).

The Raf-like kinase D-sub family was discovered for its response under salt stress in cotton. Functional validation of *GhRAF42* genes showed that this gene is involved in cotton tolerance to salt stress. This gene increased salt tolerance by regulating the genes network related to the activity of antioxidant enzymes like SOD and CAT. However, the role of *GhRAF42* needs more investigation to be used as an ideal candidate for salt-tolerant breeding (Peng *et al.*, 2021). Heat shock transcription factors (HSTFs) have been studied for their regulatory role against several abiotic stresses, especially heat stress. Rehman *et al.* (2021a) conducted a detailed analysis of HSTFs under salt stress in cotton. Expression analysis showed that *GarHSF04* was actively involved in salt tolerance in cotton. These findings lay a foundation for understanding the molecular mechanism of salinity tolerance in cotton regulated by *GarHSF04*, which activates several gene networks linked with salinity tolerance (Rehman *et al.*, 2021a). In a recent study, 384 and 257 TFs were identified in cotton exposed to salt stress. Most of the significantly expressed TFs belonged to the AP2/ERF-ERF family and were mainly expressed in roots and shoots. Some of these genes participated in plant hormone signaling pathways, and MAPK signaling pathways or networks in roots (Liu *et al.*, 2022b).

Another TF family, WRKY, is well known for its role in salinity stress; however, little is known about its role in cotton. Zhou *et al.* (2015) studied the functional characterization of WRKY member *GhWRKY34*. Overexpression of this protein increased salt tolerance in transgenic *Arabidopsis* and induced gene expression. *GhWRKY34* was involved in the Na⁺/K⁺ homeostasis pathway as transgenic lines showed a lower ratio of Na⁺/K⁺ ions. *GhWRKY34* also increased stress-related gene expression (Zhou *et al.*, 2015). *GhANN1*, a member of the ANNEXINs family of proteins, increased salt tolerance by ABA accumulation, ions homeostasis, and synthesis of phenylpropanoid in cotton. These pathways could be further studied to understand salt tolerance in cotton better (Zhang *et al.*, 2021a). Chen *et al.* (2021) identified 44 ZAT genes from the cotton genome. *GhZAT6* was expressed in root tissues under salt treatment (NaCl). Transgenic lines with overexpression of *GhZAT6* showed vigorous growth compared to the wild type (Chen *et al.*, 2021). This

gene also regulates ROS-related gene expression networks and pathways (Chen *et al.*, 2021). These studies showed the significance of protein families and their role in salinity tolerance in cotton. Several gaps were not covered in previous studies. The detailed molecular analysis of DREB, NAC, and MYB is missing, which should be covered in future research studies. These studies would increase the genetic resources useful for salt-tolerant breeding in cotton.

Table 3. Different TFs detected for salinity tolerance in cotton

TFs	Protein	Role	References
PLATZ (Plant AT-rich sequences and zinc-binding proteins)	<i>GbiPLATZ17</i> and <i>GbiPLATZ22</i>	Increase salt tolerance in cotton	(Rehman <i>et al.</i> , 2024)
TCP	<i>GbTCP4</i>	Regulate salt responses in an ABA-dependent manner	(Wang <i>et al.</i> , 2022b)
bZIP	<i>GbABF3</i>	Reduced level of cellular oxidation	(Zhang <i>et al.</i> , 2022)
C ₂ H ₂ zinc finger proteins	<i>GbZAT34</i> and <i>GbZAT79</i>	Increased seed germination rate	(Rehman <i>et al.</i> , 2021b)
KNOX	<i>GbKNOX2</i>	Enhanced salt tolerance at the seedling stage	(Zhang <i>et al.</i> , 2021c)
Raf-like kinase D-sub family	<i>GbRAF42</i>	Increased salt tolerance	(Peng <i>et al.</i> , 2021)
HSTFs	<i>GarHSF04</i>	Increased salt tolerance	(Rehman <i>et al.</i> , 2021a)
ANNEXINs	<i>GbANN1</i>	Regulated ABA accumulation	(Zhang <i>et al.</i> , 2021a)
ZAT	<i>GbZAT6</i>	Increased growth under salt stress	(Chen <i>et al.</i> , 2021)
bZIP	<i>GbABF2</i>	Improved antioxidant function	(Liang <i>et al.</i> , 2016)
WRKY	<i>GbWRKY34</i>	Decreased salt ions in transgenic plants	(Zhou <i>et al.</i> , 2015)

Transcriptome analysis and its application in salt tolerance

To clarify the molecular mechanism for salinity tolerance in cotton, it is essential to investigate the genetic makeup of the crop (Wu *et al.*, 2024; Yang *et al.*, 2024b; Zahra *et al.*, 2024). Transcriptome analysis is a significant research tool for identifying the potential gene and their regulatory network in crop salinity tolerance (Alamholo and Tarinejad, 2024; Kang *et al.*, 2024; Zhu *et al.*, 2024). Akbar *et al.* (2022) conducted a transcriptome study to determine the gene network controlling salinity tolerance in cotton. A total of 566 differentially expressed genes were identified by transcriptome analysis (Akbar *et al.*, 2022). RNA sequencing has been effective for transcriptome analysis of salt tolerance in cotton. The effect of salt treatment on gene expression profile gives us insights into the tolerance mechanism of a gene expressed under salt stress conditions. A total of 3391 and 2826 DEGs were detected in salt-treated samples before and after protoplast dissociation. These genes were enriched to several molecular pathways, including stress response and cellular macromolecule metabolic process, as shown by gene ontology (GO) analysis (Liu *et al.*, 2022b). Transcriptome-wide methyl adenosine (M⁶A) profiling and comparative analysis of cotton root tissues were performed under salt stress conditions. 3360 and 3771 m⁶A peaks involved in 3219 and 3566 DEGs genes under salt and normal conditions were identified. Results showed that the cotton YTH gene *GbECT6* improved the salt tolerance in

cotton seedlings. This data provides critical insights into the role of mRNA modification in response to salt stress (Wang *et al.*, 2022a). Cyclic nucleotide-gated ion channels (CNGCs) and calcium-permeable channels play a key role in plant development, signaling, and environmental stress. 114 CNGC genes were detected from the genome of 14 cotton species. Transcriptome data showed that some of these were induced in response to salt stress. *GhCNGC32* and *GhCNGC35* significantly improved salt tolerance in cotton (Lu *et al.*, 2022).

Aquaporins (AQPs) are vital in transporting water and small molecules across membranes and are involved in responses to environmental stresses. Guo *et al.* (2022a) identified 111, 54, and 56 AQP genes in a cotton genome and classified them further into five families. Transcriptome analysis revealed the expression of *GhPIPs* and *GhTIPs* genes in salt stress conditions. *GhPIP2:7* played a regulatory role in salt tolerance (Guo *et al.*, 2022a). Wei *et al.* (2017) conducted a transcriptome analysis for salt tolerance in cotton. The cotton plants were exposed to salt stress, and the data revealed that 8,312 and 6,732 DEGs were involved in stress tolerance in roots and leaves. Gene function annotation and expression analysis revealed the positive role of genes in ion homeostasis and oxidative balance. These results broadened our understanding of the salt tolerance mechanism in cotton (Wei *et al.*, 2017). Transcriptome analysis of two cotton cultivars showed that the *GhNHX1* gene showed higher expression in the salt-tolerant variety, L24, and the response is mainly governed by the vacuolar sequestration of Na⁺ (Guo *et al.*, 2019). Documentation of genes involved in salt tolerance at the seedling stage is essential because of the sensitivity of seedlings to salt stress. *GhERF12* ethylene response factor gene increased the salinity tolerance in cotton during early development. Different responses of cotton genotypes against salt stress show a contrasting genetic mechanism, which helps identify salt-tolerant genotypes (Zhang *et al.*, 2021b). Wang *et al.* (2021a) screened the screened salt-tolerant cultivar Zhong 9835 for transcriptome studies using different levels of Na₂SO₄ treatment. 3329 differentially expressed genes (DEGs) were identified in roots, stems, and leaves. Gene functional annotation analysis showed genes' role in scavenging ROS and ion toxicity. Identifying the genes network will provide a valuable clue for breeding salt tolerance in cotton (Wang *et al.*, 2021a).

The combined study of cotton under multiple abiotic stresses revealed the diverse mechanism of abiotic stress tolerance in cotton. 78 transcripts were identified for salinity tolerance in cotton seedlings under NaCl treatment. These genes were involved in multiple pathways to control the salinity tolerance in cotton (Zhu *et al.*, 2013). RNA-sequencing has been performed to identify the plant response to salt stress, but using sodium bicarbonate (NaHCO₃) is rarely reported. In an earlier experiment, next-generation sequencing (NGS) was applied to identify the transcriptomes involved in salinity tolerance in cotton. A total of 18,230 and 11,177 DEGs were identified in the roots and leaves of cotton. These genes were associated with the biosynthesis of hormones and hormonal signal transduction, as the Kyoto Encyclopedia of Genes and Genomes (KEGG) shows. Secondary metabolites were regulated to enhance the cotton's tolerance to salt stress (Fan *et al.*, 2021). Transcriptome analysis for identifying ROS genes is critical to mitigating the toxic effects of salt stress. Transcriptome analysis identified the 515, 260, and 261 ROS genes in different cotton genotypes. Some genes like CSD1, APX1, and APX2 played a key role in fiber development, while the ROS network-mediated pathway increased the regulation of abiotic stress in cotton (Xu *et al.*, 2019). Cotton genotypes showed different responses to different salt types. The next-generation RNA-sequence technique investigated the cotton response to different salt stresses. In response to salt stress, 25,929 and 6,564 DEG genes were identified in roots and leaves. Genes upregulated under different salt stresses were involved in ionic homeostasis; starch and sucrose metabolism give valuable insights into the genetic mechanism of salt tolerance in cotton (Zhang *et al.*, 2018). Xu *et al.* (2020) identified 41,053 DEGs in cotton plants treated with salt and cold stress, and the numbers of genes expressed under cold stress were slightly higher than under salt stress. RALF-like protein-coding gene was found in the potential shared network (Xu *et al.*, 2020).

The combined study of these molecular tools helps to get more insights into the genetic regulation of salt tolerance. RNA-seq and protein data screening results identified the 63 and 85 genes (Table 4) and proteins

under salt stress in cotton genotypes (Peng *et al.*, 2018). The comprehensive presentation and analysis of transcriptome studies will give the researchers a better understanding of salt tolerance to enhance the salt breeding process. Exploring all available cotton germplasm to screen for salt-tolerant genes using transcriptome analysis is critical.

Table 4. Potential transcriptome and their function in salt tolerance

Genes	Functions	References
444 upregulated and 354 downregulated genes	Some genes are linked with the biosynthesis of phytohormones and signaling, which includes ethylene (ET) and jasmonic acid (JA)	(Li <i>et al.</i> , 2025)
15,476 genes	Genes linked to peroxidase, transferase activity, peroxidation, plant hormone single transduction, and phenylpropanoid biosynthesis.	(Yu <i>et al.</i> , 2024)
2776, 6680, 4660, and 4174 DGEs in salt-tolerant and salt-sensitive genotypes	Many of these genes belong to starch and sucrose metabolism, plant-pathogen interaction, plant hormonal signaling, and photosynthesis.	(Peng <i>et al.</i> , 2023)
212 GhHMA	<i>GhHMA26</i> enhanced the elongation of leaf trichomes	(Wu <i>et al.</i> , 2023a)
566 genes	Significantly control the salinity tolerance	(Akbar <i>et al.</i> , 2022)
3391 and 2826	Response to stress and cellular macromolecule metabolic process	(Liu <i>et al.</i> , 2022b)
3219 and 3566	<i>GbECT6</i> improved the salt tolerance. mRNA modification plays a key role in salt tolerance	(Wang <i>et al.</i> , 2022a)
114 CNGC	<i>GbCNGC32</i> and <i>GbCNGC35</i> increased salt tolerance	(Lu <i>et al.</i> , 2022)
111, 54, and 56 AQPs genes	<i>GbPIP2:7</i> had an important role in salt tolerance	(Guo <i>et al.</i> , 2022a)
<i>GbERF12</i>	Improved salinity tolerance during early development	(Zhang <i>et al.</i> , 2021b)
3329	Scavenging of ROS	(Wang <i>et al.</i> , 2021a)
18,230 and 11,177	Regulation of secondary metabolites increased salt tolerance	(Fan <i>et al.</i> , 2021)
41,053	RALF-like protein involved in potential shared network	(Xu <i>et al.</i> , 2020)
515, 260, and 261 ROS genes	CSD1, APX1, and APX2 had role in fiber development	(Xu <i>et al.</i> , 2019)
<i>GbNHX1</i>	Promoted the vacuolar sequestration of Na ⁺	(Guo <i>et al.</i> , 2019)
25,929 and 6,564	Ionic homeostasis and sucrose metabolism	(Zhang <i>et al.</i> , 2018)
63 and 85 genes/proteins	Mitochondria are involved in energy metabolism	(Peng <i>et al.</i> , 2018)
8,312 and 6,732	Homeostasis and oxidative balance	(Wei <i>et al.</i> , 2017)
778	Involved in multiple stress-related pathways	(Zhu <i>et al.</i> , 2013)

Transgenic breeding for salt tolerance in cotton

Transgenic breeding technology is a powerful way to enhance salt tolerance and crop growth (Lian *et al.*, 2024; Yu *et al.*, 2023), but its success depends on the availability of effective genes. Understanding the molecular mechanism of plant response to salt stress is essential to employ the salt breeding technique (Table 5). Cotton lines overexpressing transgene showed enhanced tolerance to salt stress. A key gene, *GbVOZ1*, improved salt tolerance in transgenic cotton by maintaining sodium ion homeostasis *via* cell ion transport (Lian *et al.*, 2024). A major gene, *ThST5*, increased the salt tolerance in transgenic cotton lines during seed germination and seedling stage compared to the wild type. Transgenic plants showed high yields under saline conditions. *ThST5* also increased the expression of antioxidant-related genes. Salt-tolerant germplasm (*ThST5* overexpressed transgenic lines) can be further studied to increase the efficiency of transgenic technology (Nazish *et al.*, 2022). Su *et al.* (2020) transferred the *GbCIPK6a* gene to develop the transgenic cotton lines. They revealed that cotton lines with overexpression of *GbCIPK6a* showed higher water absorption capacity and seed germination. The tolerant line (*GbCIPK6a* overexpressed line) of *Gossypium arboreum* showed salt tolerance and reduced ROS activity (Su *et al.*, 2020). A transgenic cotton line overexpressing *pGSMT2g* and *ApDMT2g* was developed using the *Agrobacterium*-mediated transformation method. Transgenic cotton showed higher relative water content and low Na⁺ under salt stress (Song *et al.*, 2018). Arabidopsis *AtEDT1/HDG11* gene significantly enhanced the salt tolerance in transgenic cotton by improving the sugar and proline content. *AtEDT1/HDG11* overexpressed transgenic lines are a novel source for transgenic breeding (Yu *et al.*, 2016).

The gene *SNAC1* of the NAC TF family was recognized in rice and overexpressed in cotton cultivar YZ1. *SNAC-1* overexpressing transgenic plants exhibited more vigorous root development and enhanced proline content than wild plants (Liu *et al.*, 2014b). High-affinity K⁺ transporters (HKT) genes are known for regulating K⁺ and Na⁺ ions in plants. Overexpression of *SbHKT1* increased seed germination, biomass, antioxidant activity, and root system compared to the wild type. Cultivating transgenic germplasm (*SbHKT1* overexpressed tolerant lines) would increase the efficiency of transgenic technology (Guo *et al.*, 2020). Che *et al.* (2019) developed transgenic cotton lines by transferring *TrSOS1* using *Agrobacterium rhizogenes* strain K599. *TrSOS1* increased the salt tolerance of cotton by maintaining higher root vigor and relative water content. Hence, *Agrobacterium rhizogenes* strain K599 may be an effective candidate for enhancing salt tolerance in transgenic hairy root composite cotton seedlings. This transgenic germplasm tolerant to salt stress provides a solid foundation for additional research studies (Che *et al.*, 2019). Developing transgenic cotton cultivars (*ThST103* overexpressed lines) greatly enhanced the cotton's ability to thrive under salt-stress conditions. The *thST103* gene enabled the transgenic cotton cultivars to germinate and grow better under salt stress than wild-type ones. This gene can be a potential candidate for salt breeding in cotton (Javaid *et al.*, 2022).

Long *et al.* (2020) developed the transgenic cotton lines by transforming the *GbNHX1* gene of plant Na⁺/H⁺ antiporters (NHXs). Silencing of the *GbNHX1* gene increased cotton sensitivity to salt stress, suggesting that it plays a crucial role in regulating salt tolerance in cotton (Long *et al.*, 2020). Recently, *GbMAP4K13* of the MAP4K gene family was studied for its role in salt tolerance in cotton. *GbMAP4K13* increased stem length in cotton under salt stress conditions (Zeng *et al.*, 2025). Genetically engineered cotton cultivars have excellent capability to thrive under salt-stress conditions. As evidenced by the above studies, GE is crucial in combating the growing threats of salt stress worldwide. Therefore, using novel molecular tools is highly recommended to use the untapped genetic resources of cotton, mainly wild type, to mine the salt-tolerant genes.

Table 5. Application of genetic engineering for salt tolerance in cotton

Gene	Role	Transformation method	References
<i>GbVOZ1</i>	Maintain sodium ion homeostasis <i>via</i> cell ion transport.	Floral tip method	(Lian <i>et al.</i> , 2024)
<i>GbMAPK3</i>	Increased germination rate and root length	<i>Agrobacterium tumefaciens</i> LBA4404	(Jian <i>et al.</i> , 2024)
<i>GbBGLU24-A</i>	Mediated ER stress <i>via</i> ERAD pathway	Floral tip method	(Cui <i>et al.</i> , 2024)
<i>TbST5</i>	Increased salt tolerance at the seedling stage	<i>Agrobacterium tumefaciens</i> strain LBA4404	(Nazish <i>et al.</i> , 2022)
<i>TbST103</i>	Improved germination and growth	<i>Agrobacterium tumefaciens</i> -mediated cotton transformation	(Javaid <i>et al.</i> , 2022)
<i>GbCIPK6a</i>	Increased seed germination rate and seedling field emergence percentage	Pollen tube-pathway method	(Su <i>et al.</i> , 2020)
<i>GbNHX1</i>	Regulates salt tolerance	<i>Agrobacterium tumefaciens</i>	(Long <i>et al.</i> , 2020)
<i>SbHKT1</i>	Increased seed germination, and antioxidant activity	<i>Agrobacterium</i> -mediated transformation	(Guo <i>et al.</i> , 2020)
<i>TrSOS1</i>	Higher root vigor	<i>Agrobacterium rhizogenes</i> strain K599	(Che <i>et al.</i> , 2019)
<i>pGSMT2g</i> and <i>ApDMT2g</i>	Higher water content and low Na ⁺	<i>Agrobacterium</i> -mediated transformation	(Song <i>et al.</i> , 2018)
<i>SNAC1</i>	Root development	<i>Agrobacterium tumefaciens</i> strain LBA4404	(Liu <i>et al.</i> , 2014b)
<i>AtEDT1/HDG11</i>	Increased sugar and proline content	<i>Agrobacterium</i> -mediated transformation	(Yu <i>et al.</i> , 2016)

CRISPR\Cas9 for salinity tolerance in cotton

Genome editing of crops plays a crucial role in crop improvement (Figure 2) for various traits (Abbas *et al.*, 2023; Dongariyal *et al.*, 2023; Liu *et al.*, 2022a; Saikia *et al.*, 2024), and it is essential for basic and applied research and hence crucial for crops (Cong *et al.*, 2013). Researchers developed the CRISPR/Cas9-based gene editing tool for precise gene editing (Jinek *et al.*, 2012). CRISPR/Cas9 system consists of single-guided RNA (sgRNA) and Cas9 protein. sgRNA specifies the gene and aligns the Cas9 with the gene. Once the sgRNA and gene hybridization occurs, Cas9 confronts the targeted gene to produce double-stranded breaks (DSB) (Puchta and Fauser, 2014). CRISPR/Cas9 is divided into two classes (1 and 2) and six types (I-VI) (Makarova *et al.*, 2011). Class I to III has been widely investigated, whereas IV and VI were newly identified. CRISPR types I, II, and V edit DNA, type VI slices RNA, and type III cuts both DNA and RNA, and the cleavage action of type IV has not yet been recognized (Wang *et al.*, 2019).

Applications of molecular tools, and salt tolerance in cotton

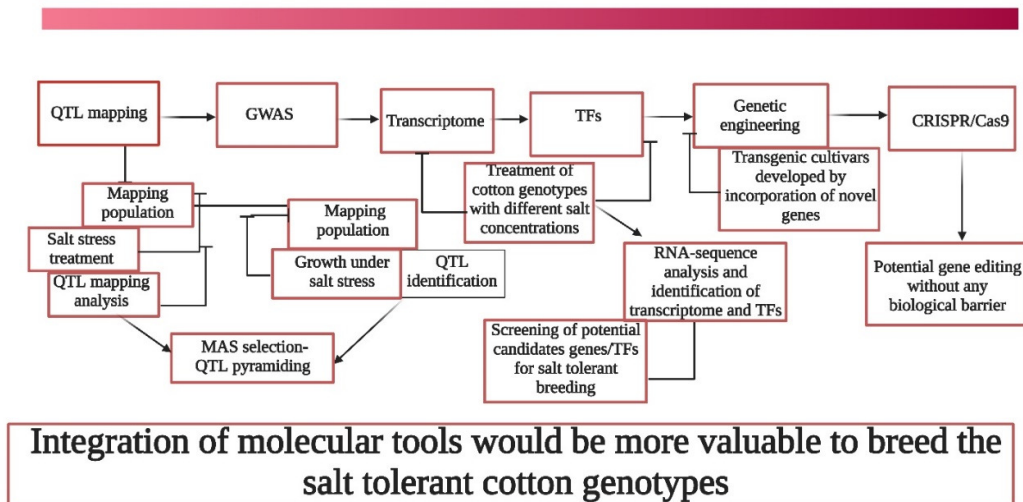


Figure 2. The employment of molecular tools is the most reliable way to uncover the genetic mechanism of salt tolerance in cotton. CRISPR/Cas9-based gene editing can speed up the salt-tolerant breeding program. QTL mapping, GWAS, transcriptome analysis, TFs analysis, and genetic engineering are potential tools to identify and transfer the genomic regions to improve salt tolerance in cotton. This figure was made with bioRender.com

Various CRISPR/Cas9-based gene editing systems, like promotor editing, multiple gene editing, and insert systems, vary in their efficiency across species (Khan *et al.*, 2023). Multiplexing allows the editing of 6-8 genes at the same time (Bortesi and Fischer, 2015). Alteration in the promotor area of the gene may change the gene expression (Čermák *et al.*, 2015). CRISPR/Cas9 has been successfully employed in many crops (Bortesi and Fischer, 2015). Recent reports suggested that the use of CRISPR/Cas9 improved salinity tolerance in major crops like rice (Zhang *et al.*, 2019) and soybean (Wang *et al.*, 2021b). There are several reports of CRISPR/Cas9 use in cotton; however, its role in salinity tolerance is rarely reported. Time is urgently needed to apply CRISPR/Cas9 to edit the gene for salt tolerance in cotton. Despite significant improvement in gene editing, there are several challenges in CRISPR/Cas9-based gene editing. CRISPR/Cas9 has significantly contributed to improvement in the last decade; however, the existing challenges still need to be overcome. Therefore, there is an urgent need to expand the use of CRISPR/Cas9 to enhance the salinity tolerance in cotton and save time and cost.

Conclusions

Salinity stress is the most devastating and challenging to mitigate among the significant abiotic factors. Salinity stress has dramatically reduced the cotton growth and yield in a large area. Cotton tolerance is polygenic; hence, conventional breeding requires laborious efforts, a long time, and cost, making it challenging to develop salt-tolerant cotton cultivars on a large scale. From the above studies, we have concluded that many efforts have been made to address the issues of salinity stress in cotton; however, despite all efforts, this issue has not yet been solved. QTL mapping, GWAS, transcriptome analysis, TFs analysis, genetic engineering, and CRISPR/Cas9 have been proven successful in identifying genes and their exploitation in salt-tolerant breeding. QTL mapping has been successfully used to explore the genetic mechanism of salinity tolerance. However, the use of QTL pyramiding is still limited, which emphasizes further studies. More efforts should be made to use

the key QTL in molecular breeding for salinity tolerance in cotton. It has been proven from the above studies that GWAS has played a key role in exploring the genetic mechanism of salinity tolerance in cotton. However, many SNPs, genes, and QTL have been identified but have yet to be used in molecular breeding. Transcriptome and TF analyses are the two most powerful research tools for identifying thousands of genes and protein families contributing to salinity tolerance in cotton. However, only a few of them are used in molecular breeding. We believe that additional studies on this aspect will provide a better understanding of salinity tolerance in cotton. One of the potent ways is to use these genes in genetic engineering to develop salt-tolerant mutants.

Transgenic technology is used to breed salt-tolerant transgenic cultivars, significantly contributing to growth and yield enhancement in cotton on saline soils. Many mutant lines have been tested for their ability to tolerate salt stress, proving the importance of genetic engineering. More studies are needed to expand the use of genetic engineering to develop salt-tolerant cotton genotypes. CRISPR/Cas9, which can revolutionize the agricultural field, is used in cotton for important agricultural traits. CRISPR-mediated gene editing has successfully edited the gene in cotton, but its role in salt tolerance has not been widely investigated. We urge to conduct more studies on this aspect and expand the potential of gene editing to reduce time and cost and to replace other molecular breeding methods. Its role should be expanded in future studies. Salinity tolerance cannot be improved by one gene technology; hence, we also suggest the integrated use of different techniques like transcriptome and metabolome as developmental tools for the detailed study of salinity tolerance in cotton. Future research studies should focus on using Unmanned Aerial Vehicle (UAV)-based phenotyping for salinity tolerance in cotton. These technologies will give a non-destructive way to collect data for various crop indexes. Wild germplasm should be exploited to identify tolerant genes for salinity tolerance in cotton. Complete genome sequencing of cotton germplasm should be completed to characterize the novel genes for salinity tolerance. By combining these efforts, we are hopeful that the genetic mechanism of salinity tolerance will be unfolded and the development of salt-tolerant cotton genotypes will be accelerated.

Authors' Contributions

Conceptualization: SW; Data curation: SW; Formal analysis: MH, HH; Funding acquisition: FAA, ZAA, SHQ; Investigation: SW, NABH; Methodology: SW; Project administration: FAA, ZAA, SHQ ; Resources: SW; Software: HH; Supervision: NABH; Validation: SW; Visualization: SW; Writing - original draft: SW; Writing - review and editing: SW, NABH.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

The authors extend their appreciation to the Deanship of Research and Graduate Studies at King Khalid University for funding this work through small- group research under grant number RGP 1/50/45.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

- Abbas A, Shah AA, Shah AN, Niaz Y, Ahmed W, Ali H, Nawaz M, Hassan MU (2023). CRISPR Revolution in Gene Editing: Targeting Plant Stress Tolerance and Physiology. In: Sustainable Agriculture in the Era of the OMICs Revolution. Springer, pp 315-325. https://doi.org/10.1007/978-3-031-15568-0_14
- Abdelraheem A, Adams N, Zhang J (2020a). Effects of drought on agronomic and fiber quality in an introgressed backcross inbred line population of Upland cotton under field conditions. *Field Crops Research* 254:107850. <https://doi.org/10.1016/j.fcr.2020.107850>
- Abdelraheem A, Fang DD, Dever J, Zhang J (2020b). QTL analysis of agronomic, fiber quality, and abiotic stress tolerance traits in a recombinant inbred population of Pima cotton. *Crop Science* 60:1823-1843. <https://doi.org/10.1002/csc2.20153>
- Abdelraheem A, Fang DD, Zhang J (2018). Quantitative trait locus mapping of drought and salt tolerance in an introgressed recombinant inbred line population of Upland cotton under the greenhouse and field conditions. *Euphytica* 214:1-20. <https://doi.org/10.1007/s10681-017-2095-x>
- Abdelraheem A, Kuraparthi V, Hinze L, Stelly D, Wedegaertner T, Zhang J (2021a). Genome-wide association study for tolerance to drought and salt tolerance and resistance to thrips at the seedling growth stage in US Upland cotton. *Industrial Crops and Products* 169:113645. <https://doi.org/10.1016/j.indcrop.2021.113645>
- Abdelraheem A, Thyssen GN, Fang DD, Jenkins JN, McCarty JC, Wedegaertner T, Zhang J (2021b). GWAS reveals consistent QTL for drought and salt tolerance in a MAGIC population of 550 lines derived from intermating of 11 Upland cotton (*Gossypium hirsutum*) parents. *Molecular Genetics and Genomics* 296:119-129. <https://doi.org/10.1007/s00438-020-01733-2>
- Akbar A, Han B, Khan AH, Feng C, Ullah A, Khan AS, He L, Yang X (2022). A transcriptomic study reveals salt stress alleviation in cotton plants upon salt tolerant PGPR inoculation. *Environmental and Experimental Botany* 200: 104928. <https://doi.org/10.1016/j.envexpbot.2022.104928>
- Alamholo M, Tarinejad A (2024). Molecular mechanism of salinity stress tolerance in barley (*Hordeum vulgare* L.) via meta-analysis of transcriptome data. *Journal of Agricultural Science and Technology* 26:111-126.
- An M, Huang X, Long Y, Wang Y, Tan Y, Qin Z, Ai X, Wang Y (2025). Salt tolerance evaluation and key salt-tolerant traits at germination stage of upland cotton. *Frontiers in Plant Science* 15:1489380. <https://doi.org/10.3389/fpls.2024.1489380>
- Anwar M, Shakeel A, Saeed A, Saleem MF (2024). Deciphering salt tolerance in cotton: unveiling insights from genetic diversity seedling stage growth parameters. *Pakistan Journal of Agricultural Sciences* 61:339-349. <https://doi.org/10.21162/PAKJAS/24.180>
- Barbosa EGG, Leite JP, Marin SRR, Marinho JP, de Fátima Corrêa Carvalho J, Fuganti-Pagliarini R, ... de Oliveira MCN (2013). Overexpression of the ABA-dependent AREB1 transcription factor from *Arabidopsis thaliana* improves soybean tolerance to water deficit. *Plant Molecular Biology Reporter* 31:719-730. <https://doi.org/10.1007/s11105-012-0541-4>
- Bortesi L, Fischer R (2015). The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnology Advances* 33:41-52. <https://doi.org/10.1016/j.biotechadv.2014.12.006>
- Čermák T, Baltes NJ, Čegan R, Zhang Y, Voytas DF (2015). High-frequency, precise modification of the tomato genome. *Genome Biology* 16: 1-15. <https://doi.org/10.1186/s13059-015-0796-9>
- Chaudhary MT, Majeed S, Rana IA, Ali Z, Jia Y, Du X, Hinze L, Azhar MT (2024). Impact of salinity stress on cotton and opportunities for improvement through conventional and biotechnological approaches. *BMC Plant Biology* 24: 20. <https://doi.org/10.1186/s12870-023-04558-4>
- Che B, Cheng C, Fang J, Liu Y, Jiang L, Yu B (2019). The recretahalophyte *Tamarix TrSOS1* gene confers enhanced salt tolerance to transgenic hairy root composite cotton seedlings exhibiting virus-induced gene silencing of *GbSOS1*. *International Journal of Molecular Sciences* 20: 2930. <https://doi.org/10.3390/ijms20122930>

- Chen G, Liu Z, Li S, Qanmber G, Liu L, Guo M, Lu L, Ma S, Li F, Yang Z (2021). Genome-wide analysis of ZAT gene family revealed *GhZAT6* regulates salt stress tolerance in *G. hirsutum*. *Plant Science* 312:111055. <https://doi.org/10.1016/j.plantsci.2021.111055>
- Chen W, Hou Z, Wu L, Liang Y, Wei C (2010). Effects of salinity and nitrogen on cotton growth in arid environment. *Plant and Soil* 326:61-73. <https://doi.org/10.1007/s11104-008-9881-0>
- Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science* 339: 819-823. [doi: 10.1126/science.1231143](https://doi.org/10.1126/science.1231143)
- Cui C, Wan H, Li Z, Ai N, Zhou B (2024). Long noncoding RNA TRABA suppresses β -glucosidase-encoding BGLU24 to promote salt tolerance in cotton. *Plant Physiology* 194: 1120-1138. <https://doi.org/10.1093/plphys/kiad530>
- Desingh R, Kanagaraj G, Nagar A (2007). Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. *General and Applied Plant Physiology* 33:221-234.
- Dilnur T, Peng Z, Pan Z, Palanga KK, Jia Y, Gong W, Du X (2019). Association analysis of salt tolerance in Asiatic cotton (*Gossypium arboreum*) with SNP markers. *International Journal of Molecular Sciences* 20:2168. <https://doi.org/10.3390/ijms20092168>
- Diouf L, Pan Z, He S-P, Gong W-F, Jia YH, Magwanga RO, Romy KRE, Or Rashid H, Kirungu JN, Du X (2017). High-density linkage map construction and mapping of salt-tolerant QTLs at seedling stage in upland cotton using genotyping by sequencing (GBS). *International Journal of Molecular Sciences* 18:2622. <https://doi.org/10.3390/ijms18122622>
- Doğan İ, Kekeç G, Özyiğit İİ, Sakçalı MS (2012). Salinity induced changes in cotton (*Gossypium hirsutum* L.). *Pakistan Journal of Botany* 44:21-25.
- Dongariyal A, Chandra AK, Dongriyal A, Kumar A, Sharma P (2023). Tending genome editing via CRISPR/Cas9-induced mutagenesis: Opportunity and challenges for yield, quality and nutritional improvement of fruit crops. *Scientia Horticulturae* 311: 111790. <https://doi.org/10.1016/j.scienta.2022.111790>
- Du L, Cai C, Wu S, Zhang F, Hou S, Guo W (2016). Evaluation and exploration of favorable QTL alleles for salt stress related traits in cotton cultivars (*G. hirsutum* L.). *PLoS One* 11:e0151076. <https://doi.org/10.1371/journal.pone.0151076>
- Fan Y, Lu X, Chen X, Wang J, Wang D, Wang S, Guo L, Rui C, Zhang Y, Cui R (2021). Cotton transcriptome analysis reveals novel biological pathways that eliminate reactive oxygen species (ROS) under sodium bicarbonate (NaHCO₃) alkaline stress. *Genomics* 113: 1157-1169. <https://doi.org/10.1016/j.ygeno.2021.02.022>
- Fang H, Gao X, Wu Y, Zhang K, Wu Y, Li J, Qian D, Li R, Gu H, Mehari TG, Shen X, Wang B (2025). Unveiling the role of *GhP5CSI* in cotton salt stress tolerance: a comprehensive genomic and functional analysis of P5CS genes. *Plants (Basel)* 14: 231. <https://doi.org/10.3390/plants14020231>
- FAO (2011). *The State of the World's Land and Water Resources for Food and Agriculture (SOLAW) — Managing Systems at Risk*. Food and Agriculture Organization of the United Nations. Rome and Earthscan, London.
- Farooq MA, Shakeel A, Zafar MM, Farooq M, Chattha WS, Husnain T (2022). A study towards the development of salt tolerant upland cotton (*Gossypium hirsutum* L.). *Journal of Natural Fibers* 19:4115-4131. <https://doi.org/10.1080/15440478.2020.1854144>
- Furihata T, Maruyama K, Fujita Y, Umezawa T, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2006). Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. *Proceedings of the National Academy of Sciences* 103:1988-1993. <https://doi.org/10.1073/pnas.0505667103>
- Ghassemi-Golezani K, Mousavi SA, Farhangi-Abri S (2024). Enriched biochars with silicon and calcium nanoparticles mitigated salt toxicity and improved safflower plant performance. *International Journal of Phytoremediation*: 26:1359-1368. <https://doi.org/10.1080/15226514.2024.2321167>
- Gu Q, Ke H, Liu C, Lv X, Sun Z, Liu Z, Rong W, Yang J, Zhang Y, Wu L (2021). A stable QTL qSalt-A04-1 contributes to salt tolerance in the cotton seed germination stage. *Theoretical and Applied Genetics* 134:2399-2410. <https://doi.org/10.1007/s00122-021-03831-0>
- Guo A-H, Su Y, Huang Y, Wang Y-M, Nie H-S, Zhao N, Hua J-P (2021). QTL controlling fiber quality traits under salt stress in upland cotton (*Gossypium hirsutum* L.). *Theoretical and Applied Genetics* 134:661-685. <https://doi.org/10.1007/s00122-020-03721-x>

- Guo A, Hao J, Su Y, Li B, Zhao N, Zhu M, Huang Y, Tian B, Shi G, Hua J (2022a). Two aquaporin genes, *GbPIP2; 7* and *GbTIP2; 1*, positively regulate the tolerance of upland cotton to salt and osmotic stresses. *Frontiers in Plant Science* 12:780486. <https://doi.org/10.3389/fpls.2021.780486>
- Guo A, Li H, Huang Y, Ma X, Li B, Du X, Cui Y, Zhao N, Hua J (2024). Yield-related quantitative trait loci identification and lint percentage hereditary dissection under salt stress in upland cotton. *The Plant Journal*. 119:115-136. <https://doi.org/10.1111/tbj.16747>
- Guo A, Su Y, Nie H, Li B, Ma X, Hua J (2022b). Identification of candidate genes involved in salt stress response at germination and seedling stages by QTL mapping in upland cotton. *G3 Genes/Genomics/Genetics* 12: jkac099. <https://doi.org/10.1093/g3journal/jkac099>
- Guo H, Li S, Min W, Ye J, Hou Z (2019). Ionic and transcriptomic analyses of two cotton cultivars (*Gossypium hirsutum* L.) provide insights into the ion balance mechanism of cotton under salt stress. *PloS One* 14:e0226776. <https://doi.org/10.1371/journal.pone.0226776>
- Guo Q, Meng S, Tao S, Feng J, Fan X, Xu P, Xu Z, Shen X (2020). Overexpression of a samphire high-affinity potassium transporter gene *SbHKT1* enhances salt tolerance in transgenic cotton. *Acta Physiologiae Plantarum* 42:1-8. <https://doi.org/10.1007/s11738-020-3027-2>
- Guo T, Wang S, Fan B, Zou S, Chen S, Liu W, Wang S, Ai L, Han L (2023). Overexpression of the *Zoysia japonica* *ZjABR1/ERF10* regulates plant growth and salt tolerance in transgenic *Oryza sativa*. *Environmental and Experimental Botany* 206:105171. <https://doi.org/10.1016/j.envexpbot.2022.105171>
- Han X, Gao C, Liu L, Zhang Y, Jin Y, Yan Q, Yang L, Li F, Yang Z (2022). Integration of eQTL analysis and GWAS highlights regulation networks in cotton under stress condition. *International Journal of Molecular Sciences* 23: 7564. <https://doi.org/10.3390/ijms23147564>
- He C, Yan J, Shen G, Fu L, Holaday AS, Auld D, Blumwald E, Zhang H (2005). Expression of an Arabidopsis vacuolar sodium/proton antiporter gene in cotton improves photosynthetic performance under salt conditions and increases fiber yield in the field. *Plant and Cell Physiology* 46:1848-1854. <https://doi.org/10.1093/pcp/pci201>
- Huang XY, Zhao FJ (2020). QTL pyramiding for producing nutritious and safe rice grains. *Journal of Integrative Plant Biology* 62:264-268. <https://doi.org/10.1111/jipb.12920>
- Huijun Z, Hezhong D, Yuejin S, Shouyi C, Yonghong Z (2010). Transformation of cotton *Gossypium hirsutum* L. with *AbCMO* gene and the expression of salinity tolerance. *Acta Agronomica Sinica* 7:1073-1078.
- Ibrahim W, Zhu YM, Chen Y, Qiu CW, Zhu S, Wu F (2019). Genotypic differences in leaf secondary metabolism, plant hormones and yield under alone and combined stress of drought and salinity in cotton genotypes. *Physiologia Plantarum* 165:343-355. <https://doi.org/10.1111/ppl.12862>
- Jamil A, Riaz S, Ashraf M, Foolad M (2011). Gene expression profiling of plants under salt stress. *Critical Reviews in Plant Sciences* 30:435-458. <https://doi.org/10.1080/07352689.2011.605739>
- Javaid A, Nazish T, Ali M, Zhu Y, Li J, Zhang H, Wu J, Xiang C, Wu S, Alfatih A (2022). *Thellungiella halophila* *ST103* enhances salt tolerance in *Gossypium hirsutum*. *Journal of Cotton Research* 5:1-11. <https://doi.org/10.1186/s42397-022-00120-z>
- Jia Y-H, Sun J-l, Wang X-W, Zhou Z-l, Pan Z-E, He S-P, Pang B-Y, Wang L-R, Du X-M (2014). Molecular diversity and association analysis of drought and salt tolerance in *Gossypium hirsutum* L. germplasm. *Journal of Integrative Agriculture* 13:1845-1853. [https://doi.org/10.1016/S2095-3119\(13\)60668-1](https://doi.org/10.1016/S2095-3119(13)60668-1)
- Jian H, Sadau SB, Wei F, Ahmad A, Lu Z, Ma L, Fu X, Zhang N, Lu J, Yin G (2024). *GhMAPK3*, a mitogen-activated protein kinase, enhance salt and drought tolerance in cotton (*Gossypium hirsutum*). *Industrial Crops and Products* 214:118492. <https://doi.org/10.1016/j.indcrop.2024.118492>
- Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 337:816-821. <https://doi.org/10.1126/science.1225829>
- Ju F, Pang J, Huo Y, Zhu J, Yu K, Sun L, Loka DA, Hu W, Zhou Z, Wang S (2021). Potassium application alleviates the negative effects of salt stress on cotton (*Gossypium hirsutum* L.) yield by improving the ionic homeostasis, photosynthetic capacity and carbohydrate metabolism of the leaf subtending the cotton boll. *Field Crops Research* 272: 108288. <https://doi.org/10.1016/j.fcr.2021.108288>

- Kang BH, Chowdhury S, Kang S-H, Shin S-Y, Lee W-H, Lee H-S, Ha B-K (2024). Transcriptome profiling of a soybean mutant with salt tolerance induced by gamma-ray irradiation. *Plants* 13:254. <https://doi.org/10.3390/plants13020254>
- Khan Z, Khan SH, Ahmed A, Iqbal MU, Mubarik MS, Ghouri MZ, Ahmad F, Yaseen S, Ali Z, Khan AA (2023). Genome editing in cotton: challenges and opportunities. *Journal of Cotton Research* 6:1-21. <https://doi.org/10.1186/s42397-023-00140-3>
- Lan Thi Hoang X, Du Nhi NH, Binh Anh Thu N, Phuong Thao N, Phan Tran L-S (2017). Transcription factors and their roles in signal transduction in plants under abiotic stresses. *Current genomics* 18:483-497. <https://doi.org/10.2174/1389202918666170227150057>
- Li H, Liu L, Kong X, Wang X, Si A, Zhao F, Huang Q, Yu Y, Chen Z (2025). Time-course transcriptomics analysis reveals molecular mechanisms of salt-tolerant and salt-sensitive cotton cultivars in response to salt stress. *International Journal of Molecular Sciences* 26:329. <https://doi.org/10.3390/ijms26010329>
- Li X, Liu X, Pan F, Hu J, Han Y, Bi R, ... Li J (2024). Dissection of major QTLs and candidate genes for seedling stage salt/drought tolerance in tomato. *BMC Genomics* 25:1170. <https://doi.org/10.1186/s12864-024-11101-8>
- Lian B, Wu A, Wu H, Lv X, Sun M, Li Y, Lu Z, Li S, An L, Guo X (2024). *GhVOZ1-APPI* module positively regulates salt tolerance in upland cotton (*Gossypium hirsutum* L.). *International Journal of Biological Macromolecules* 258:129116. <https://doi.org/10.1016/j.ijbiomac.2023.129116>
- Liang C, Meng Z, Meng Z, Malik W, Yan R, Lwin KM, Lin F, Wang Y, Sun G, Zhou T (2016). *GhABF2*, a bZIP transcription factor, confers drought and salinity tolerance in cotton (*Gossypium hirsutum* L.). *Scientific reports* 6:1-14. <https://doi.org/10.1038/srep35040>
- Liu C, Mao B, Ou S, Wang W, Liu L, Wu Y, Chu C, Wang X (2014a). *OsbZIP71*, a bZIP transcription factor, confers salinity and drought tolerance in rice. *Plant Molecular Biology* 84:19-36. <https://doi.org/10.1007/s11103-013-0115-3>
- Liu G, Li X, Jin S, Liu X, Zhu L, Nie Y, Zhang X (2014b). Overexpression of rice NAC gene *SNAC1* improves drought and salt tolerance by enhancing root development and reducing transpiration rate in transgenic cotton. *PLoS One* 9: e86895. <https://doi.org/10.1371/journal.pone.0086895>
- Liu H, Chen W, Li Y, Sun L, Chai Y, Chen H, Nie H, Huang C (2022a). CRISPR/Cas9 technology and its utility for crop improvement. *International Journal of Molecular Sciences* 23:10442. <https://doi.org/10.3390/ijms231810442>
- Liu Q, Li P, Cheng S, Zhao Z, Liu Y, Wei Y, Lu Q, Han J, Cai X, Zhou Z (2022b). Protoplast dissociation and transcriptome analysis provides insights to salt stress response in cotton. *International Journal of Molecular Sciences* 23: 2845. <https://doi.org/10.3390/ijms23052845>
- Liu Q, Li P, Umer MJ, Abbas M, Zhao Y, Chen Y, ... Peng R (2025). Identification of *EXPA4* as a key gene in cotton salt stress adaptation through transcriptomic and coexpression network analysis of root tip protoplasts. *BMC Plant Biology* 25:65. <https://doi.org/10.1186/s12870-024-05958-w>
- Long L, Yang W-W, Liao P, Guo Y-W, Kumar A, Gao W (2019). Transcriptome analysis reveals differentially expressed ERF transcription factors associated with salt response in cotton. *Plant Science* 281:72-81. <https://doi.org/10.1016/j.plantsci.2019.01.012>
- Long L, Zhao J-R, Guo D-D, Ma X-N, Xu F-C, Yang W-W, Gao W (2020). Identification of NHXs in *Gossypium* species and the positive role of *GbNHX1* in salt tolerance. *BMC Plant Biology* 20:1-13. <https://doi.org/10.1186/s12870-020-02345-z>
- Lu Z, Yin G, Chai M, Sun L, Wei H, Chen J, Yang Y, Fu X, Li S (2022). Systematic analysis of *CNGCs* in cotton and the positive role of *GbCNGC32* and *GbCNGC35* in salt tolerance. *BMC Genomics* 23:1-13. <https://doi.org/10.1186/s12864-022-08800-5>
- Luo X, Wu J, Li Y, Nan Z, Guo X, Wang Y, Zhang A, Wang Z, Xia G, Tian Y (2013). Synergistic effects of *GhSOD1* and *GhCAT1* overexpression in cotton chloroplasts on enhancing tolerance to methyl viologen and salt stresses. *PLoS One* 8:e54002. <https://doi.org/10.1371/journal.pone.0054002>
- Lv S, Zhang K, Gao Q, Lian L, Song Y, Zhang J (2008). Overexpression of an H⁺-PPase gene from *Thellungiella halophila* in cotton enhances salt tolerance and improves growth and photosynthetic performance. *Plant and Cell Physiology* 49: 1150-1164. <https://doi.org/10.1093/pcp/pcn090>

- Makarova KS, Haft DH, Barrangou R, Brouns SJ, Charpentier E, Horvath P, Moineau S, Mojica FJ, Wolf YI, Yakunin AF (2011). Evolution and classification of the CRISPR–Cas systems. *Nature Reviews Microbiology* 9:467-477. <https://doi.org/10.1038/nrmicro2577>
- Maryum Z, Luqman T, Nadeem S, Khan SMUD, Wang B, Ditta A, Khan MKR (2022). An overview of salinity stress, mechanism of salinity tolerance and strategies for its management in cotton. *Frontiers in Plant Science* 13: 907937. <https://doi.org/10.3389/fpls.2022.907937>
- Meloni DA, Oliva MA, Ruiz HA, Martinez CA (2001). Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. *Journal of Plant Nutrition* 24:599-612. <https://doi.org/10.1081/PLN-100104983>
- Muhammad N, Dong Q, Luo T, Zhang X, Song M, Wang X, Ma X (2025). New developments in understanding cotton's physiological and molecular responses to salt stress. *Plant Stress*: 100742. <https://doi.org/10.1016/j.stress.2025.100742>
- Murtaza G, Ahmed Z, Iqbal R, Deng G (2025). Biochar from agricultural waste as a strategic resource for promotion of crop growth and nutrient cycling of soil under drought and salinity stress conditions: a comprehensive review with context of climate change. *Journal of Plant Nutrition* 1-52. <https://doi.org/10.1080/01904167.2025.2460769>
- Nazish T, Javaid A, Ali M, Zhu Y, LI J, Zhang H, Wu J, Xiang C, Wu S, Alfatih A (2022). *Tbellungiella halophila* ST5 improves salt tolerance in cotton. *Journal of Cotton Research* 5:1-12. <https://doi.org/10.1186/s42397-022-00112-z>
- Nieves-Cordones M, Al Shiblawi FR, Sentenac H (2016). Roles and Transport of Sodium and Potassium in Plants. *Metal Ions in Life Science*. Springer, Cham 16: 291-324. https://doi.org/10.1007/978-3-319-21756-7_9
- Oluoch G, Zheng J, Wang X, Khan MKR, Zhou Z, Cai X, Wang C, Wang Y, Li X, Wang H (2016). QTL mapping for salt tolerance at seedling stage in the interspecific cross of *Gossypium tomentosum* with *Gossypium hirsutum*. *Euphytica* 209:223-235. <https://doi.org/10.1007/s10681-016-1674-6>
- Peng Z, He S, Gong W, Xu F, Pan Z, Jia Y, Geng X, Du X (2018). Integration of proteomic and transcriptomic profiles reveals multiple levels of genetic regulation of salt tolerance in cotton. *BMC Plant Biology* 18:1-19. <https://doi.org/10.1186/s12870-018-1350-1>
- Peng Z, Jiang X, Wang Z, Wang X, Li H, He S, Pan Z, Qayyum A, Rehman A, Du X (2021). Identification of Raf-Like kinases B subfamily genes in gossypium species revealed *GhRAF42* enhanced salt tolerance in cotton. *International Journal of Molecular Sciences* 22:12649. <https://doi.org/10.3390/ijms222312649>
- Peng Z, Rehman A, Jiang X, Tian C, Wang Z, Li H, Wang X, Ahmad A, Azhar MT, Du X (2025). Comparative transcriptome analysis and functional verification revealed that *GhSAP6* negatively regulates salt tolerance in upland cotton. *Plant Physiology and Biochemistry* 219: 109406. <https://doi.org/10.1016/j.plaphy.2024.10940>
- Peng Z, Rehman A, Li X, Jiang X, Tian C, Wang X, Li H, Wang Z, He S, Du X (2023). Comprehensive evaluation and transcriptome analysis reveal the salt tolerance mechanism in semi-wild cotton (*Gossypium purpurascens*). *International Journal of Molecular Sciences* 24: 12853. <https://doi.org/10.3390/ijms241612853>
- Phour M, Sindhu SS (2023). Soil salinity and climate change: microbiome-based strategies for mitigation of salt stress to sustainable agriculture. In "Climate Change and Microbiome Dynamics", *Climate Change Management*. Springer, Cham. https://doi.org/10.1007/978-3-031-21079-2_13
- Puchta H, Fauser F (2014). Synthetic nucleases for genome engineering in plants: prospects for a bright future. *The Plant Journal* 78:727-741. <https://doi.org/10.1111/tpj.12338>
- Rasheed A, Raza A, Jie H, Mahmood A, Ma Y, Zhao L, Xing H, Li L, Hassan MU, Qari SH (2022). Molecular tools and their applications in developing salt-tolerant soybean (*Glycine max* L.) cultivars. *Bioengineering* 9:495. <https://doi.org/10.3390/bioengineering9100495>
- Rehman A, Atif RM, Azhar MT, Peng Z, Li H, Qin G, Jia Y, Pan Z, He S, Qayyum A (2021a). Genome wide identification, classification and functional characterization of heat shock transcription factors in cultivated and ancestral cottons (*Gossypium* spp.). *International Journal of Biological Macromolecules* 182:1507-1527. <https://doi.org/10.1016/j.ijbiomac.2021.05.016>
- Rehman A, Tian C, Li X, Wang X, Li H, He S, Jiao Z, Qayyum A, Du X, Peng Z (2024). *GhiPLATZ17* and *GhiPLATZ22*, zinc-dependent DNA-binding transcription factors, promote salt tolerance in upland cotton. *Plant Cell Reports* 43:140. <https://doi.org/10.1007/s00299-024-03178-y>

- Rehman A, Wang N, Peng Z, He S, Zhao Z, Gao Q, Wang Z, Li H, Du X (2021b). Identification of C2H2 subfamily ZAT genes in *Gossypium* species reveals *GbZAT34* and *GbZAT79* enhanced salt tolerance in *Arabidopsis* and cotton. *International Journal of Biological Macromolecules* 184:967-980. <https://doi.org/10.1016/j.ijbiomac.2021.06.166>
- Saddique MAB, Ali Z, Sher MA, Farid B, Ahmad F, Arshad SF (2022). Gene Mapping in Cotton. In: *Cotton Breeding and Biotechnology*. CRC Press, pp 69-85.
- Saeed M, Wangzhen G, Tianzhen Z (2014). Association mapping for salinity tolerance in cotton (*Gossypium hirsutum* L.) germplasm from US and diverse regions of China. *Australian Journal of Crop Science* 8:338-346.
- Saikia B, S R, Debbarma J, Maharana J, Sastry GN, Chikkaputtaiah C (2024). CRISPR/Cas9-based genome editing and functional analysis of *SHyPRP1* and *SIDEA1* genes of *Solanum lycopersicum* L. in imparting genetic tolerance to multiple stress factors. *Frontiers in Plant Science* 15:1304381. <https://doi.org/10.3389/fpls.2024.1304381>
- Shahbaz M, Ashraf M (2013). Improving salinity tolerance in cereals. *Critical Reviews in Plant Sciences* 32:237-249. <https://doi.org/10.1080/07352689.2013.758544>
- Shakir AM, Geng M, Tian J, Wang R (2025). Dissection of QTLs underlying the genetic basis of drought resistance in wheat: a meta-analysis. *Theoretical and Applied Genetics* 138:1-28. <https://doi.org/10.1007/s00122-024-04811-w>
- Sharif I, Aleem S, Farooq J, Rizwan M, Younas A, Sarwar G, Chohan SM (2019). Salinity stress in cotton: effects, mechanism of tolerance and its management strategies. *Physiology and Molecular Biology of Plants* 25:807-820. <https://doi.org/10.1007/s12298-019-00676-2>
- Shehzad M, Shafeeq-ur-rahman DA, Iqbal M, Malik T, Hussain S, Khan M, Ramzan M (2019a). Effect of salinity stress on Cotton growth and role of marker assisted breeding and agronomic practices (chemical, biological and physical) for salinity tolerance. *Scholars Reports* 4:1-14.
- Sikder RK, Xiang-ru W, Heng-heng Z, Hui-ping G, Qiang D, Ding-sha J, Mei-zhen S (2022). Influence of nitrogen on the growth and yield of cotton under salinity stress. *Journal of Plant Nutrition* 45:1181-1197. <https://doi.org/10.1080/01904167.2021.1994598>
- Song J, Zhang R, Yue D, Chen X, Guo Z, Cheng C, Hu M, Zhang J, Zhang K (2018). Co-expression of *ApGSMT2g* and *ApDMT2g* in cotton enhances salt tolerance and increases seed cotton yield in saline fields. *Plant Science* 274:369-382. <https://doi.org/10.1016/j.plantsci.2018.06.007>
- Su Y, Geng S, Guo A, Ijaz B, Li B, Chen Q, Zheng K, Chen Q, Hua J (2021). Genetic dissection of QTLs linked with seedling, yield and fiber quality traits of upland cotton under salt stress field conditions. *Euphytica* 217:1-20. <https://doi.org/10.1007/s10681-021-02947-0>
- Su Y, Guo A, Huang Y, Wang Y, Hua J (2020). *GbCIPK6a* increases salt tolerance in transgenic upland cotton by involving in ROS scavenging and MAPK signaling pathways. *BMC Plant Biology* 20:1-19. <https://doi.org/10.1186/s12870-020-02548-4>
- Sun F, Chen Q, Chen Q, Jiang M, Qu Y (2023). Yield-based drought tolerance index evaluates the drought tolerance of cotton germplasm lines in the interaction of genotype-by-environment. *PeerJ* 11:e14367. <https://doi.org/10.7717/peerj.14367>
- Sun H, Meng M, Yan Z, Lin Z, Nie X, Yang X (2019). Genome-wide association mapping of stress-tolerance traits in cotton. *The Crop Journal* 7: 77-88. <https://doi.org/10.1016/j.cj.2018.11.002>
- Sun Z, Li H, Zhang Y, Li Z, Ke H, Wu L, Zhang G, Wang X, Ma Z (2018). Identification of SNPs and candidate genes associated with salt tolerance at the seedling stage in cotton (*Gossypium hirsutum* L.). *Frontiers in Plant Science* 9:1011. <https://doi.org/10.3389/fpls.2018.01011>
- Tiwari RS (2012). "Germplasm evaluation and mapping of candidate genes and quantitative trait loci for salt tolerance in a backcross inbred population of cotton. New Mexico State University.
- Wang F, Wang L, Zou X, Duan S, Li Z, Deng Z, Luo J, Lee SY, Chen S (2019). Advances in CRISPR-Cas systems for RNA targeting, tracking and editing. *Biotechnology Advances* 37:708-729. <https://doi.org/10.1016/j.biotechadv.2019.03.016>
- Wang Q, Lu X, Chen X, Malik WA, Wang D, Zhao L, Wang J, Wang S, Guo L, Cui R (2021a). Transcriptome analysis of upland cotton revealed novel pathways to scavenge reactive oxygen species (ROS) responding to Na₂SO₄ tolerance. *Scientific Reports* 11:1-15. <https://doi.org/10.1038/s41598-021-87999-x>

- Wang T, Xun H, Wang W, Ding X, Tian H, Hussain S, Dong Q, Li Y, Cheng Y, Wang C (2021b). Mutation of *GmA1TR* genes by CRISPR/Cas9 genome editing results in enhanced salinity stress tolerance in soybean. *Frontiers in Plant Science* 12:779598. <https://doi.org/10.3389/fpls.2021.779598>
- Wang W, Li W, Cheng Z, Sun J, Gao J, Li J, Niu X, Amjid MW, Yang H, Zhu G (2022a). Transcriptome-wide N6-methyladenosine profiling of cotton root provides insights for salt stress tolerance. *Environmental and Experimental Botany* 194:104729. <https://doi.org/10.1016/j.envexpbot.2021.104729>
- Wang Y, Yu Y, Wan H, Tang J, Ni Z (2022b). The sea-island cotton *GbTCP4* transcription factor positively regulates drought and salt stress responses. *Plant Science*: 111329. <https://doi.org/10.1016/j.plantsci.2022.111329>
- Wei Y, Xu Y, Lu P, Wang X, Li Z, Cai X, Zhou Z, Wang Y, Zhang Z, Lin Z (2017). Salt stress responsiveness of a wild cotton species (*Gossypium klotzschianum*) based on transcriptomic analysis. *PLoS One* 12:e0178313. <https://doi.org/10.1371/journal.pone.0178313>
- Wu C, Xiao S, Zuo D, Cheng H, Zhang Y, Wang Q, Lv L, Song G (2023a). Genome-wide analysis elucidates the roles of GhHMA genes in different abiotic stresses and fiber development in upland cotton. *Plant Physiology and Biochemistry* 194:281-301. <https://doi.org/10.1016/j.plaphy.2022.11.022>
- Wu D, Chen C, Liu Y, Yang L, Yong JWH (2023b). Iso-osmotic calcium nitrate and sodium chloride stresses have differential effects on growth and photosynthetic capacity in tomato. *Scientia Horticulturae* 312: 111883. <https://doi.org/10.1016/j.scienta.2023.111883>
- Wu K, Wang X, Zhang H, Tian L, Luo H, Chen Y, Liang Q, Ma Y, Bate N (2023c). Responses of cotton seed germination characteristics and storage substance metabolism to saline-alkali stress. *Research Square* <https://doi.org/10.21203/rs.3.rs-2414291/v1>
- Wu Z, Meng R, Feng W, Wongsansilp T, Li Z, Lu X, Wang X (2024). Study of Dandelion (*Taraxacum mongolicum* Hand.-Mazz.) Salt response and caffeic acid metabolism under saline stress by transcriptome analysis. *Genes* 15:220. <https://doi.org/10.3390/genes15020220>
- Xu P, Guo Q, Meng S, Zhang X, Xu Z, Guo W, Shen X (2021). Genome-wide association analysis reveals genetic variations and candidate genes associated with salt tolerance related traits in *Gossypium hirsutum*. *BMC Genomics* 22:1-14. <https://doi.org/10.1186/s12864-020-07321-3>
- Xu Y, Magwanga RO, Cai X, Zhou Z, Wang X, Wang Y, Zhang Z, Jin D, Guo X, Wei Y (2019). Deep transcriptome analysis reveals reactive oxygen species (ROS) network evolution, response to abiotic stress, and regulation of fiber development in cotton. *International Journal of Molecular Sciences* 20:1863. <https://doi.org/10.3390/ijms20081863>
- Xu Y, Magwanga RO, Jin D, Cai X, Hou Y, Juyun Z, Agong SG, Wang K, Liu F, Zhou Z (2020). Comparative transcriptome analysis reveals evolutionary divergence and shared network of cold and salt stress response in diploid D-genome cotton. *BMC Plant Biology* 20:1-17. <https://doi.org/10.1186/s12870-020-02726-4>
- Xue T, Li X, Zhu W, Wu C, Yang G, Zheng C (2009). Cotton metallothionein *GhMT3a*, a reactive oxygen species scavenger, increased tolerance against abiotic stress in transgenic tobacco and yeast. *Journal of Experimental Botany* 60:339-349. <https://doi.org/10.1093/jxb/ern291>
- Yamaguchi-Shinozaki K, Shinozaki K (1994). A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *The Plant Cell* 6:251-264. <https://doi.org/10.1105/tpc.6.2.251>
- Yan Q, Zhang J, Li X, Wang Y (2019). Effects of salinity stress on seed germination and root growth of seedlings in island cotton. *Acta Agronomica Sinica* 45:100-110.
- Yang X, Bai Z, He Y, Wang N, Sun L, Li Y, Yin Z, Wang X, Zhang B, Han M (2024a). Genome-wide characterization of DNA methyltransferase family genes implies *GbDMT6* improving tolerance of salt and drought on cotton. *BMC Plant Biology* 24:1-16. <https://doi.org/10.1186/s12870-024-04985-x>
- Yang X, Liu D, Liu C, Li M, Yan Z, Zhang Y, Feng G (2024b). Possible melatonin-induced salt stress tolerance pathway in *Phaseolus vulgaris* L. using transcriptomic and metabolomic analyses. *BMC Plant Biology* 24:72. <https://doi.org/10.1186/s12870-023-04705-x>
- Yasir M, He S, Sun G, Geng X, Pan Z, Gong W, Jia Y, Du X (2019). A genome-wide association study revealed key SNPs/genes associated with salinity stress tolerance in upland cotton. *Genes* 10:829. <https://doi.org/10.3390/genes10100829>

- Yu H, Guo Q, Ji W, Wang H, Tao J, Xu P, Chen X, Ali W, Wu X, Shen X (2024). Transcriptome expression profiling reveals the molecular response to salt stress in *Gossypium anomalum* seedlings. *Plants* 13:312. <https://doi.org/10.3390/plants13020312>
- Yu LH, Wu SJ, Peng YS, Liu RN, Chen X, Zhao P, Xu P, Zhu JB, Jiao GL, Pei Y (2016). Arabidopsis EDT 1/HDG 11 improves drought and salt tolerance in cotton and poplar and increases cotton yield in the field. *Plant Biotechnology Journal* 14:72-84. <https://doi.org/10.1111/pbi.12358>
- Yu Z, Duan X, Luo L, Dai S, Ding Z, Xia G (2020). How plant hormones mediate salt stress responses. *Trends in Plant Science* 25:1117-1130. <https://doi.org/10.1016/j.tplants.2020.06.008>
- Yu Z, Niu L, Cai Q, Wei J, Shang L, Yang X, Ma R (2023). Improved salt-tolerance of transgenic soybean by stable over-expression of *AbBADH* gene from *Atriplex hortensis*. *Plant Cell Reports* 42:1291-1310. <https://doi.org/10.1007/s00299-023-03031-8>
- Yuan Y, Xing H, Zeng W, Xu J, Mao L, Wang L, Feng W, Tao J, Wang H, Zhang H (2019). Genome-wide association and differential expression analysis of salt tolerance in *Gossypium hirsutum* L at the germination stage. *BMC Plant Biology* 19:1-19. <https://doi.org/10.1186/s12870-019-1989-2>
- Zafar MM, Ijaz A, Anwar Z, Iqbal MS, Zafar S, Subhan M, Seleiman MF, Alhammad BA, Aljabri M, Qiao F (2025). Harnessing genetic diversity in cotton for enhanced resilience against salt stress by using agro-physiological characters. *Plant Production Science* 28:51-68. <https://doi.org/10.1080/1343943X.2024.2439874>
- Zafar MM, Shakeel A, Haroon M, Manan A, Sahar A, Shoukat A, Mo H, Farooq MA, Ren M (2022). Effects of salinity stress on some growth, physiological, and biochemical parameters in cotton (*Gossypium hirsutum* L.) germplasm. *Journal of Natural Fibers* 19:8854-8886. <https://doi.org/10.1080/15440478.2021.1975596>
- Zahra N, Uzair M, Zaid IU, Attia KA, Inam S, Fiaz S, Abdallah RM, Naeem MK, Farooq U, Rehman N (2024). The comparative transcriptome analysis of two green super rice genotypes with varying tolerance to salt stress. *Molecular Biology Reports* 51:22. <https://doi.org/10.1007/s11033-023-08998-x>
- Zeb S, Sana J, Hafsa K, Ayesha F (2016). Effect of salt stress on growth and antioxidant enzymes in two cultivars of maize (*Zea mays* L.). *Pakistan Journal of Botany* 48:1361-1370.
- Zeng Q, Peng F, Wang J, Wang S, Lu X, Bakhsh A, Li Y, Qaraevna BZ, Ye W, Yin Z (2025). Identification of the MAP4K gene family reveals *GhMAP4K13* regulates drought and salt stress tolerance in cotton. *Physiologia Plantarum* 177:e70031. <https://doi.org/10.1111/ppl.70031>
- Zhang A, Liu Y, Wang F, Li T, Chen Z, Kong D, Bi J, Zhang F, Luo X, Wang J (2019). Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the *OsRR22* gene. *Molecular Breeding* 39:1-10. <https://doi.org/10.1007/s11032-019-0954-y>
- Zhang B, Chen X, Lu X, Shu N, Wang X, Yang X, Wang S, Wang J, Guo L, Wang D (2018). Transcriptome analysis of *Gossypium hirsutum* L. reveals different mechanisms among NaCl, NaOH and Na₂CO₃ stress tolerance. *Scientific Reports* 8:1-14. <https://doi.org/10.1038/s41598-018-31668-z>
- Zhang D, Li J, Niu X, Deng C, Song X, Li W, Cheng Z, Zhang B, Guo W (2021a). *GhANN1* modulates the salinity tolerance by regulating ABA biosynthesis, ion homeostasis and phenylpropanoid pathway in cotton. *Environmental and Experimental Botany* 185:104427. <https://doi.org/10.1016/j.envexpbot.2021.104427>
- Zhang H, Mao L, Xin M, Xing H, Zhang Y, Wu J, Xu D, Wang Y, Shang Y, Wei L (2022). Overexpression of *GhABF3* increases cotton (*Gossypium hirsutum* L.) tolerance to salt and drought. *BMC Plant Biology* 22:1-15. <https://doi.org/10.1186/s12870-022-03705-7>
- Zhang J, Zhang P, Huo X, Gao Y, Chen Y, Song Z, Wang F, Zhang J (2021b). Comparative phenotypic and transcriptomic analysis reveals key responses of upland cotton to salinity stress during post germination. *Frontiers in Plant Science* 12:639104. <https://doi.org/10.3389/fpls.2021.639104>
- Zhang X, Zhao J, Wu X, Hu G, Fan S, Ma Q (2021c). Evolutionary relationships and divergence of KNOTTED1-Like family genes involved in salt tolerance and development in cotton (*Gossypium hirsutum* L.). *Frontiers in Plant Science* 12:774161. <https://doi.org/10.3389/fpls.2021.774161>
- Zhao Y, Wang H, Shao B, Chen W, Guo Z, Gong H, Sang X, Wang J, Ye W (2016). SSR-based association mapping of salt tolerance in cotton (*Gossypium hirsutum* L.). *Genetics and Molecular Research* 15:15027370. <http://doi.org/10.4238/gmr.15027370>

- Zheng J, Zhang Z, Gong Z, Liang Y, Sang Z, Xu Y, Li X, Wang J (2021). Genome-wide association analysis of salt-tolerant traits in terrestrial cotton at seedling stage. *Plants* 11:97. <https://doi.org/10.3390/plants11010097>
- Zhou H, Shi H, Yang Y, Feng X, Chen X, Xiao F, Lin H, Guo Y (2023). Insights into plant salt stress signaling and tolerance. *Journal of Genetics and Genomics* 51:16-34. <https://doi.org/10.1016/j.jgg.2023.08.007>
- Zhou L, Wang N-N, Gong S-Y, Lu R, Li Y, Li X-B (2015). Overexpression of a cotton (*Gossypium hirsutum*) WRKY gene, *GhWRKY34*, in *Arabidopsis* enhances salt-tolerance of the transgenic plants. *Plant Physiology and Biochemistry* 96: 311-320. <https://doi.org/10.1016/j.plaphy.2015.08.016>
- Zhu G, Gao W, Song X, Sun F, Hou S, Liu N, Huang Y, Zhang D, Ni Z, Chen Q (2020). Genome-wide association reveals genetic variation of lint yield components under salty field conditions in cotton (*Gossypium hirsutum* L.). *BMC Plant Biology* 20:1-13. <https://doi.org/10.1186/s12870-019-2187-y>
- Zhu W, Ren X, Liu Z, Li Y, Li G, Wang J, Li D, Shi Y, Wang C, Sun H (2024). Identification of salt-responsive genes in melatonin regulated alfalfa salt tolerance by transcriptome analysis. *Journal?* <https://doi.org/10.20944/preprints202403.1815.v2>
- Zhu Y-N, Shi D-Q, Ruan M-B, Zhang L-L, Meng Z-H, Liu J, Yang W-C (2013). Transcriptome analysis reveals crosstalk of responsive genes to multiple abiotic stresses in cotton (*Gossypium hirsutum* L.). *PloS One* 8:e80218. <https://doi.org/10.1371/journal.pone.0080218>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



License - Articles published in *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.
© Articles by the authors; Licensee UASVM and SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.

Notes:

- **Material disclaimer:** The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- **Maps and affiliations:** The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- **Responsibilities:** The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.