



Innovative and Sustainable Cultivation Techniques for Enhancing Abiotic Stress Tolerance and Efficiency in Cotton Production: Comprehensive Review

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Abstract

One of the world's biggest crops, cotton (*Gossypium* spp.) is liked for its oilseed and textile goods. However, abiotic stressors such as dry period, salinity, tropical heat and heavy metal toxicity continue to pose problems for its farming. These stressors diminish metabolic and physiological processes which remarkably reduce the yield and quality. This study looks at innovative and environmentally responsible ways to increase cotton's resistance to abiotic stresses and boost production efficiency. Understanding physiological and molecular mechanisms specifically, the functions of transcription factors like WRKY and MYB heat shock proteins and ABA and ROS signaling pathways that help to lessen the consequences of stress is one of the significant progress. Breeding efforts supported by quantitative trait loci (QTL) mapping and marker-assisted selection are being complemented by the use of modern biotechnological technologies like as RNA interference, transgenic genome editing using the CRISPR/Cas system, and multi-omics approaches. Agronomic techniques that have been shown to be successful in stabilizing cellular structures and improving photosynthetic performance under stress include potassium supplementation, growth regulators and osmoprotectants. Targeting intricate stress-response circuits, cutting-edge technologies such as transient gene expression systems and CRISPR base editing allow for precise genetic alterations. Despite these evolution, creating cost-effective, stress-tolerant cotton cultivars still faces several barriers. The importance of multidisciplinary approaches that combine molecular biology traditional breeding and sustainable farming practices is by this review. In order to adjust cotton production to the reality of climate change and limited resources and eventually make it a more robust and stable agricultural commodity such all-encompassing techniques are important.

Keywords: *Climate resilience, CRISPR/Cas genome editing, Potassium fertilization, Growth regulators, Stress-responsive mechanisms, Transgenic.*

1. Introduction

An indispensable fiber crop cultivated worldwide, cotton (*Gossypium* spp.) is fighting growing threats to its quality and productivity due to abiotic stressors and climate change (Kopecka, *et al.*, 2023). Due to these stressors, cotton yields have notably lessen worldwide creating major production issues. Such decreases can have significant

global economic outcome due to the crop's vital role in the manufacture of consumable oil and textiles (Jabran, *et al.*, 2020).

The main factors determining cotton fertility are abiotic stresses, which include radiation, heavy metals, drought, salt and extreme temperatures (both high and low). These

factors all throw the plant's regular growth and metabolic processes (Bita and Gerats, 2013). Pathogens, invasive plants and pests are examples of biotic variables that also lead to yield losses. Abiotic stressors usually have a greater effect with yield losses of up to 50%, whereas biotic stressors cause production decreases of 10–30%. Losses of up to 50–60% may result from the overall effects of biotic stressors and drought (Noreen, *et al.*, 2020). Especially, harmful are high temperatures especially those exceeding 36 °C, which reduce photosynthesis compromise membrane stability and mediate with metabolic processes. Important proteins and enzymes like catalase and superoxide dismutase (SOD) are deactivated in these conditions to prevent oxidative stress and maintain metabolic activities in cotton plants (Pilon, *et al.*, 2018). Drought stress further reduce turgor pressure, which has an effect on photosynthesis, carbohydrate metabolism, boll retention and leaf size both directly and indirectly (Snider, *et al.*, 2014). According to research, creating novel technologies and breeding cotton varieties that can resist stress are important for reducing the effects of various ecological stresses. These strategies are essential for improving cotton's resistance to severe environmental factors such as desiccation, salt stress, intense heat and guaranteeing its sustainability in the face of unsuitable circumstances (Zahoor, *et al.*, 2017).

Cotton's ability to withstand stress and increase yield has been greatly amplified by traditional breeding techniques. However, the need for cotton cultivars with sophisticated physiological systems to permit abiotic stresses is expanding due to the complexity of various stress factors. There are stimulating opportunities for creating stress-resilient cotton by combining contemporary molecular tools with breeding techniques. With an emphasis on water desiccation, heat, heavy metals and flooding in the context of a changing climate, highlights recent developments in our understanding of cotton physiology under abiotic stresses and their effects on yield and quality. In order to produce stress-tolerant cultivars it also

investigates biotechnological methods like genome editing, transgenics, RNA interference and multi-omics techniques. These instruments facilitate the quicker creation of flexible, stress-resistant crops by offering a deeper understanding of the biochemical and metabolic alterations that take place in cotton under stress. In order to face the challenges presented by a variety of environmental circumstances and ensure the long range viability of growing cotton it is imperative that the genetic underpinnings of stress tolerance be addressed.

2. Cotton Plant Mechanism in Abiotic Stress-Signaling Routes

Several physiological and morphological adjustments, like, stomatal regulation efficient reduced leaf water loss, photosynthetic activity, maintenance of high relative water content, osmotic adjustment and the development of deeper and enlarged taproots are among the morpho-physiological adaptations that help cotton withstand drought (Ahmad, *et al.*, 2022). Plants detect drought stress signals, which trigger hormone-dependent and self-activated signaling pathways that often involve mitogen-activated protein kinase (MAPK) cascades. In these signaling pathways, calcium ions (Ca²⁺) often operate as second messengers, combining with hormones like ethylene, abscisic acid and jasmonic acid. In particular, ABA is essential because it triggers physiological and biochemical reactions to drought stress by working through SnRK2 proteins (Liu, *et al.*, 2017). Diminishing the negative impacts of drought stress requires improving physio-morphological features (Zafar, *et al.*, 2023).

Hormone interactions are crucial for adjusting how plants react to complex stress situations (Seleiman, *et al.*, 2021). Under abiotic stress, transcription factors including, ERF, DREB, MYB and HSF are significant gene expression regulators that govern the activation or suppression of target genes (Wang, *et al.*, 2019). These transcriptional alterations set off a chain reaction of gene activity that results in physiological and biochemical adaptations,

such as the formation of chaperones, antioxidant enzymes and osmolytes. These process aid plants in conserving cellular homeostasis, safeguarding cellular components and improving their ability to face cruel environmental circumstances. The

complex interaction between genetic pathways and hormonal signals in constructing stress resistance is reflected in the upstream activation of stress-responsive genes, which results in tolerance to abiotic stress (Baillo, et al., 2019).

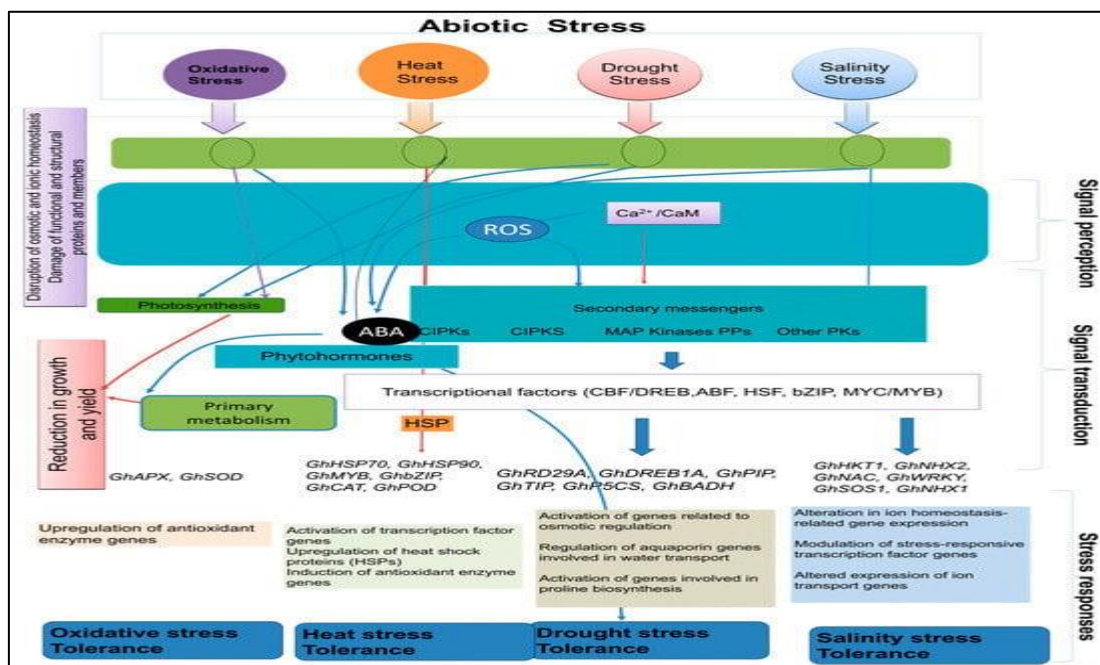


Figure 1: The signaling routes and response of abiotic stress mechanisms in cotton are outlined (Patil et al., 2024)

Figure1. The signaling routes and response of abiotic stress mechanisms in cotton are outlined. Complex molecular, biochemical and physiological mechanisms enable cotton to adapt to abiotic stressors. The ABA signaling system controls ABA synthesis and improves defiance to drought and salinity. It is composed of stress-responsive genes (GhANN1), transcription factors (CaHB12), ABA receptors (GhPYL9-5D, GhPYR1-3A). While antioxidant enzymes like CAT, POD, reduce oxidative stress and SOD amplify

resistance reactive oxygen species (ROS) have a dual role in stress responses by acting as signaling molecules and causing damage. GhHSP70-26 and GhHRP are examples of HSPs that increasing resistance to heat and drought. Furthermore, calcium signaling via MAPK cascades (GhMPK3, GhMAPKK5) and calcium-binding proteins (GhCBL3) regulates stress responses which helps cotton resist a variety of environmental stresses (Patil, et al., 2024).

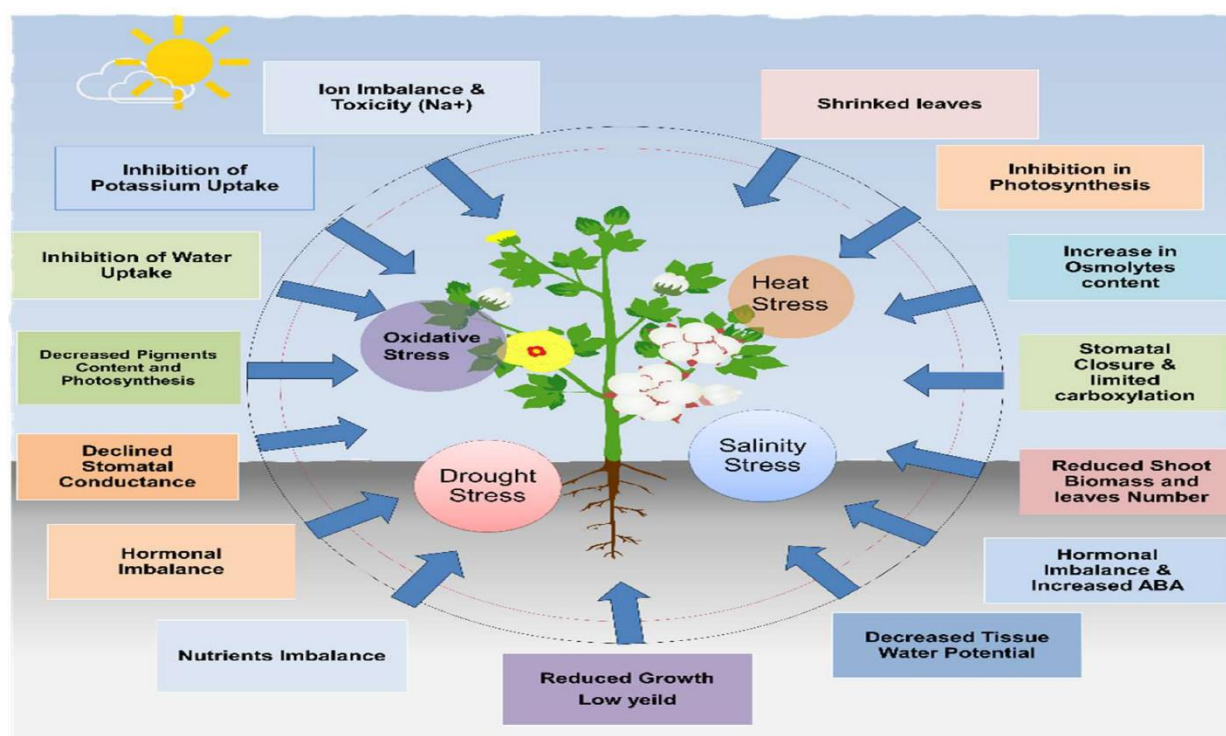


Figure 2: The effect of stress regarding the cotton plant physiology (Patil, *et al.*, 2024)

2.1 Roles of Genes in the ABA Signaling Pathway in Cotton's Response to Abiotic Stress

Cotton's response to abiotic stressors is enormously influenced by the abscisic acid signaling system, which mainly aid closure of stomata to conserves water and triggers gene activation that improve stress tolerance. The development of cotton's tolerance to drought and stress depends on a number of genes engaged in ABA-dependent pathways. By controlling ion homeostasis, ABA biosynthesis and phenylpropanoid pathway. Cotton's stress reactions and detoxifying procedures are also outstandingly influenced by WRKY. The significance of transcriptional factor WRKY genes in cotton's ability to adapt to shifting environmental conditions is further highlighted by their interplay with ABA signaling (Abid, *et al.*, 2018). Highlighted how the stress signaling network in cotton is intricate by the interaction of WRKY transcription factors with ethylene and abscisic acid. The plant's response to various challenges relies on the interplay between WRKY proteins and the ABA signaling route (Xiong, *et al.*, 2020). Studies indicate that overexpression of GhANN1 increases ABA levels and enhances the expression of stress-

responsive genes, with a particular emphasis on its interaction with GhWRKY40-like proteins. This interaction plays a vital role in strengthening cotton's resistance to salt stress (Wasilewska, *et al.*, 2008).

By increasing photosynthetic efficiency and water consumption, modifying Abscisic acid dependent pathways and lowering indole-3-acetic acid levels to stop leaf shedding. CaHB12 improves resistance to drought. CaHB12's function in intensifying water scarcity tolerance is illustrated by transgenic cotton that overexpresses important ABA signaling genes (Zhang, *et al.*, 2021). In response to PEG treatment, the ABA receptors GhPYR1-3A and GhPYL9-5D show enhanced expression, co-expressing with transcription factors, redox signaling and auxin signals components.

By combining several signaling pathways, they play an essential role in Cotton's resistance to osmotic stress and salinity (Basso, *et al.*, 2021). By controlling genes related to drought responses and affecting ABA production, GhMYB102 also contributes to drought resistance in cotton. Furthermore, cotton's drought response depends on the GhMPPK32, GhMCKK16, GhMAP3K62, MAPK

cascade, regulates ABA dependent stomatal movement and stimulates ABA production helping the plant in surviving drought stress (Liu, et al., 2023). Moreover, studies have shown that mepiquat chloride priming enhances the cottonseed's ability to endure salt stress by activating GABA signaling which in turn regulates the ascorbate glutathione cycle (Chen, et al., 2023).

2.2 The Role of Heat Shock Protein in Cotton's ability to Handle Stress

Because they protect cellular structures, prevent protein aggregation under stress, maintain folding of proteins properly, HSPs are crucial for cotton's survival under abiotic stress. One important factor in enhancing the resistance of cotton to drought is the 70-kDa HSPs GhHSP70-26. Stress causes this protein's expression to rise and tobacco plants that overexpress it have demonstrated increased stress tolerance. Cotton, on the other hand, is less resilient to stress when GhHSP70-26 is inhibited (Qi, et al., 2023). Additionally, studies have encountered the biochemical and genetic processes underlying cotton's reactions to heat stress (Maqbool, et al., 2009).

By increasing gene expression during water desiccation, a 360 bp insertion in the GhHSP70-26 gene's promoter region has been found to be a crucial component in enhancing drought tolerance. It has been demonstrated that selecting this gene increases cottons adaptation to drought (Ijaz, et al., 2024). Furthermore, by controlling hormone signaling and shielding chloroplasts, the heat-responsive protein GhHRP is essential for heat tolerance. GhHRP improves cotton's resistance to heat stress in conjunction with the GhPIF4/GhEIN3 complex (Guo, et al., 2023). According to these results, HSPs in particular, GhHSP70-26 and GhHRP are crucial targets for creating cotton cultivars with increased stress tolerance in order to tackle the challenges presented by a changing environment.

2.3 Stress-Related Genes and Proteins

Several abiotic stressors are making crop production more difficult. Abscisic acid (ABA), which frequently interacts with both

routes of ABA is an essential part of the intricate signaling pathways and mechanisms that plants have developed to deal with a variety of difficulties. Understanding the molecular mechanisms underlying stress responses is essential for breeding cotton types that can withstand a range of environmental difficulties and enhance yield. The differential expression of genes that respond to abiotic stress is shown in Table 1. The draft genome sequencing of allotetraploid cotton is one example of how genomic advances have tremendously benefited the study of stress-responsive genes. *G. hirsutum* L. TM-1 and diploid cotton *G. raimondii* (Abdullah, et al., 2022). Keen understanding of the molecular mechanisms behind cotton's stress responses can be gained by using this genomic data to examine stress-related pathways (Wang, et al., 2012), this information will help recognize genes that can resist stress and allow for targeted genetic changes for cotton that will enhance its outcome under a variety of abiotic stress settings.

Improving crop water usage efficiency has appeared as a major area of study for cotton, especially when it comes to drought adaption techniques. It is generally known that WRKY transcription factors contribute to regulating how plants react to abiotic stress. (Zhang, et al., 2015). These elements are essential for the transmission of stress signals and have a major impact on how model plants and crops react to stress. The complex interaction of transcription factors in response to both stresses in cotton and other plants is spotlighted by the WRKY one of the most well-studied gene families implicated in stress related processes (Banerjee, et al., 2015).

For instance, GhWRKY15 contributes to plant development and disease resistance highlighting the many functions of WRKY transcription factors. These elements are essential for controlling cotton's stress reactions and detoxification highlighting how important they are to the plant's capacity to adapt to environmental changes (Xiong, et al., 2020). The cotton stress signaling network is further complicated by the interaction of

WRKY proteins with ethylene and ABA (Wasilewska, *et al.*, 2008). The way that plants react to various stimuli is greatly influenced by this relationship (Basso, *et al.*, 2021). Two essential steps in starting the entire stress response in plants are the production of ROS and the activation of transcription factors.

Table 1. An outline of the ways in which abiotic stress causes a variety of gene expression. The tabular data contains information on genes, corresponding the abiotic stressors, impacted plant tissues, the effect on the regulation of gene expression the study methods and other information sources (Patil, *et al.*, 2024).

Table 1: Cotton Genotype for biochemical traits under different stress conditions

Biochemical traits	Effects on biochemical traits	Explant source	Screening method
Proline accumulation	Increased under drought stress, indicative of osmotic adjustment	Leaf tissue	High-performance liquid chromatography (HPLC)
Chlorophyll content	Decreased under heat stress, indicating photoinhibition	Leaf tissue	HPLC
Antioxidant enzyme activity	Enhanced activity under oxidative stress, protecting against damage	Leaf tissue	Enzyme Assays
Ion homeostasis	Alterations in nutrient uptake, essential for plant growth	Root tissue	Ion Analysis
Heat shock protein expression	Induced expression under high temperatures, aiding protein stability	Leaf tissue	Protein Analysis
Peroxidase (pod) activity	Changes in metabolic pathways, impacting plant growth and development	Root tissue	Enzyme Assays
Lipid peroxidation	Increased levels indicate membrane damage under stress conditions	Leaf tissue	Thiobarbituric Acid Assay
Soluble sugar content	Accumulation acts as an osmoprotectant, maintaining cellular integrity	Leaf tissue	Spectrophotometry
Total phenolic content	Elevated levels contribute to antioxidant defense against stress	Leaf tissue	Spectrophotometry
Malondialdehyde (mda) content	Elevated levels indicate lipid peroxidation and cellular damage	Leaf tissue	Spectrophotometry
Superoxide dismutase (sod) activity	Increased activity under oxidative stress, scavenging superoxide radicals	Leaf tissue	Enzyme Assays
Catalase (cat) activity	Enhanced activity under oxidative stress, decomposing hydrogen peroxide	Leaf tissue	Enzyme Assays
Carotenoid content	Decreased levels impact photosynthetic efficiency under stress	Leaf tissue	HPLC
Flavonoid content	Increased synthesis contributes	Leaf	Spectrophotometry

	to stress tolerance mechanisms	tissue	
Ascorbic acid content	Decreased levels affect antioxidant capacity and stress tolerance	Leaf tissue	Titration Method
Glutathione content	Altered levels impact oxidative stress response and redox regulation	Leaf tissue	Enzymatic Assay
Polyphenol oxidase activity	Enhanced activity in response to stress, leading to tissue browning	Leaf tissue	Enzyme Assay

3. Methods of Breeding and Biotechnology to Increase Cotton's Tolerance to Abiotic Stress

3.1 Tolerance for Stress in Breeding

The primary method for creating cotton cultivars with improved stress tolerance has always been breeding. Cotton's resistance to abiotic stressors has been greatly enhanced by recent breeding developments. The main closure from genetic research molecular marker applications and physiological reactions pertaining to cotton's ability to withstand abiotic stress are assembled in this article. The heritability of drought-resistant characteristics for example, has been better understood thanks to genetic research of desiccation tolerance in *G. barbadense* L. The genetic factors governing resistance to salinity and drought have been the subject of much research (Kushanov, et al., 2020). Developing more resilient cotton cultivars requires integrating tolerance to many stresses (Mwando, et al., 2020). The creation of genetic maps and the discovery of quantitative trait loci linked to enhanced stress tolerance have been made easier by marker-assisted selection (Han, et al., 2022).

The identification of key QTLs underlying cotton's resistance to abiotic stress has been made possible in large part by genome-wide association studies (GWAS) (Shukla, et al., 2021). These examinations have identified genomic areas in upland cotton that are connected to osmotic stress and salt tolerance. These regions might be intended for MAS in breeding initiatives (Sun, et al., 2023). Furthermore, according to a GWAS, upland cotton populations' stress both biotic and abiotic tolerance markers have been found

(Olouch, et al., 2016). Cotton germplasm's resistance to salinity and drought has been the subject of more research which has made it easier to conserve and exploit genetic resources with many degrees of tolerance (Said, et al., 2013). The valuable information on the genetic basis of stress tolerance provided by QTL mapping research has bolstered the adoption of marker-assisted breeding approaches. The possibility of using wild cotton species in breeding programs is demonstrated by the discovery of QTLs tolerance for salt through interspecific crosses of *G. tomentosum* and *G. hirsutum* (Li, et al., 2015).

With Regard to plant genetic engineering, acquiring genes is still a major obstacle. However, there is a chance to find new genes that are activated during stress in sequencing of entire genome and omics technologies as metabolomics, proteomics and genomics (Li, et al., 2023). Enhancing plant stress tolerance may be greatly aided by these recently identified genes. Finding metabolites in crops linked to stress is also crucial for creating resilient plants. A more effective method is gene pyramiding which entails the simultaneous expression of multiple functionally related genes as overexpressing individual genes often leads to less stress tolerance (He, et al., 2023).

3.2 CRISPR and Cas in Cotton

The groundbreaking CRISPR (clustered regularly interspaced short palindromic repeats) technologies have altered genome editing (Yan, et al., 2022). New opportunities for precise genetic modifications centered on stress response pathways have been made possible by recent applications of

CRISPR/Cas in cotton, especially in species whose whole genome sequencing has been completed (Shelake, *et al.*, 2022). The post-genomic age highlighted the need for efficient biotechnological tools for studying gene functions, reverse omics and genetics based approaches (Meladinovic, *et al.*, 2021). Gene silencing techniques, such as RNA interference have been widely employed since the early 2000s to study the role and regulatory mechanisms of genes. However, given the shortcomings of existing gene-silencing technologies editing is a useful tool with possibilities beyond stable and inheritable modifications (Gaj, *et al.*, 2013).

Several genome editing (GE) methods exist including zinc finger nucleases, CRISPR/Cas and transcription activator-like effector nucleases (Javaid, *et al.*, 2022). Among these, the CRISPR/Cas system, which makes use of small RNA molecules called single guide RNAs has revolutionized plant genetic engineering due to its high efficiency and ease of usage. This approach has resolved the complexity and challenges of designing and cloning desired gene constructs that are characteristic of ZFNs and TALENs (Misra, *et al.*, 2024). The preferred method for GE in plants is CRISPR/Cas since it is inexpensive and easy to utilize which has revolutionized genetic research. Cotton a vital crop for the production of fiber, ethanol and oil greatly benefits from this state-of-the-art tool for comprehensive gene functional study (Li, *et al.*, 2017).

Despite its remarkable success in genetically modifying a variety of crops and model organisms, the CRISPR/Cas system has not consistently produced results in cotton due to a number of issues, including the polyploidy of cotton and drawn-out, intricate transformation processes. Using genes like GhCLA1, GhARG, GhVP and MYB25. For instance, a recent study successfully used the CRISPR/Cas system to generate plants with the GhNAC3 gene deletion (Shelake, *et al.*, 2024). GhNAC3 encodes a transcription factor from the NAC family that acts independently and in conjunction with abscisic acid to

promote drought stress tolerance. The gene's function in overall growth, development and drought stress resistance was shown by the phenotypic alterations in the mutant lines.

Furthermore, the precise nucleotide substitutions enabled by CRISPR/Cas-based base editing enable targeted genomic changes and guided evolution of specific loci. The application of base editing in cotton GE has been investigated by several research teams. (Wang and others, 2019).

Transient gene expression plays a crucial part in understanding abiotic stress tolerance processes and developing strategies to mitigate the impact of such challenges. To overcome the challenges posed by long transformation processes in cotton a fast method for sgRNA validation for CRISPR based genetic engineering was developed (Makarova, *et al.*, 2018). The effectiveness of this technique has been shown in a variety of applications such as the validation of sgRNAs for genes like GhEF1 and GhPDS the simultaneous editing of homologous genes, and the deletion of genomic segments. Temporary expression in the cotyledons allows for rapid experimental validation in cotton plants. This method has proven versatile and has been successfully applied to produce CRISPR/Cas-induced mutations including those that target GhCLA1 in stable genetic transformations exhibited unique albino traits. (Zhang, *et al.*, 2021). Our strategy expands the use of CRISPR/Cas-mediated methods in cotton research and applications by eliminating the trouble of drawn-out transformation approaches and providing a quick validation platform for sgRNAs.

Understanding gene function has been greatly enhanced by the combination of focused genetic engineering approaches and reverse genetics. According to the most recent GMO laws, CRISPR/Cas-based methods in particular make it easier to create transgene-free plants by backcrossing or self-pollination (Perez-Clemente, *et al.*, 2013). As shown in other crops, the potential use of CRISPR for genome-wide modifications in cotton offers promising opportunities to enhance genetic

traits, improve disease resistance, optimize agronomic characteristics and boost overall productivity (Lv, *et al.*, 2008). Because of its great efficiency and ease of application, CRISPR/Cas has become an essential tool for broad gene functional study in cotton (Yan, *et al.*, 2022). As genetic engineering technology

advances it is expected that sophisticated tools like base editing and prime editing will offer deeper insights into gene functions leading to innovative cotton breeding methods including the development of cultivars with greater resilience to abiotic stresses.

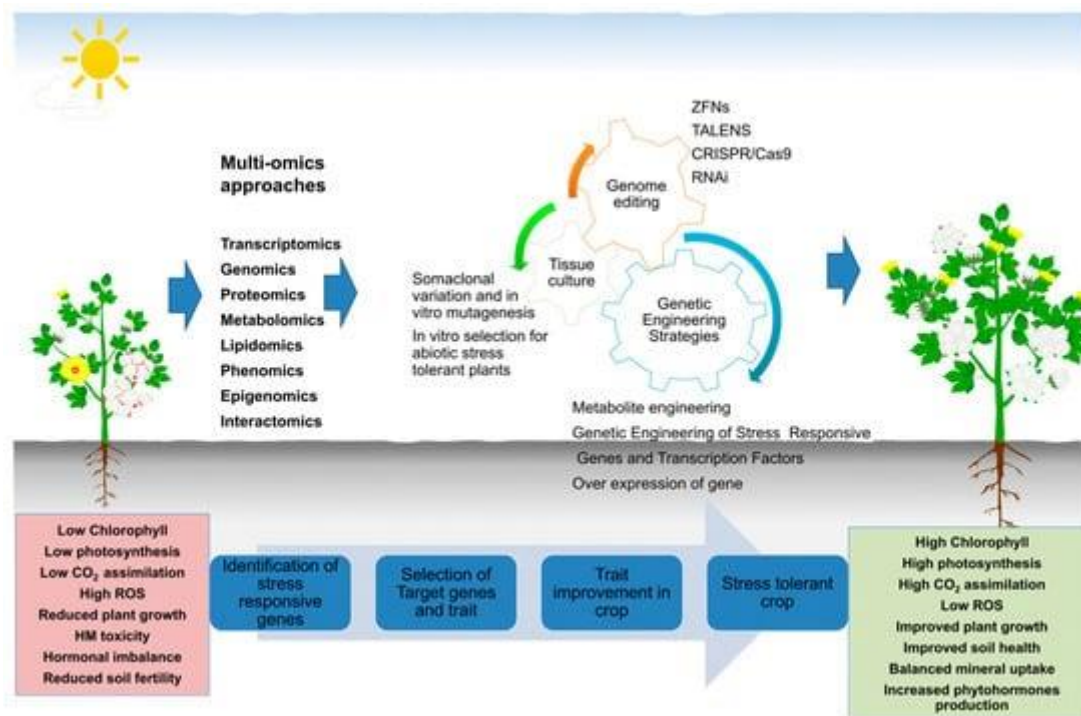


Figure 3: The combination of reverse genetics and targeted genetic engineering techniques has significantly improved our understanding of gene function (Yan *et al.*, 2022)

Figure 3 The combination of reverse genetics and targeted genetic engineering techniques has significantly improved our understanding of gene function. The latest GMO regulations state that CRISPR based techniques in particular facilitate the creation of plants without transgene by self-pollination (Perez-Clemente, *et al.*, 2013). As shown in other crops, the prospective application of CRISPR/Cas for genome wide changes in cotton presents encouraging prospects to boost genetic features raise resistance to diseases improve agronomic qualities and increase productivity. It has emerged as a crucial tool for comprehensive gene function studies in cotton due to its high efficiency and simplicity (Yan, *et al.*, 2022). With advancements in genetic engineering, innovative techniques such as base editing and prime editing are anticipated to provide deeper understanding of gene functions. This

might lead to innovative cotton breeding methods such producing cultivars that are more resilient to abiotic stressors (Patil, *et al.*, 2024).

3.3 Transgenic Methods

Recent advances in genetic engineering to enhance cotton's fortitude to abiotic stress have been vitally examined in order to better understand these strategies. Researchers have used a range of genetic engineering techniques to solve abiotic stress concerns. This article provides a comprehensive analysis of genetic engineering methods for enhancing cotton cultivars Capable of withstanding abiotic stresses. Incorporating drought-responsive genes through genetic modification has notably enhanced drought tolerance in transgenic cotton, highlighting possible approaches for developing stress-

resistant cultivars by targeting critical genes associated with stress responses.

By modifying the expression of genes and transcriptional factor that react to dryness, drought resistance can be increased. A genome-wide analysis of the CDPK gene family in *Gossypium raimondii* has revealed an intricate pathways involved in stress signaling underscoring the potential of genetic engineering to improve cotton's tolerance to environmental challenges. Similarly, it has been discovered that cotton's improved resilience to salinity and drought is mostly due to the bZIP transcription factor GhABF2 (Lisei-De-Ss, *et al.*, 2017). Research has shown that overexpressing the rice NAC1 gene in cotton enhances its resistance to adverse conditions, supporting the potential of cross-species gene transfer for improving stress resilience. Similarly, the H⁺-PPase gene from *Thellungiella halophila* has been found to significantly increase growth, salt tolerance, and photosynthetic efficiency when overexpressed, underscoring its role in improving cotton's performance under abiotic stress conditions.

Cotton fiber elongation and improved drought resistance depend on the gene GhCDPK1 and the calcium sensor GhCaM7. Cotton that overexpresses genes like AtDRE2A-CA and GhABF2 has also been demonstrated to be more resistant to abiotic stress (Lv, *et al.*, 2008). The development of cotton with enhanced stress tolerance cultivars necessitates improvements in our knowledge of transcription factors and root traits like MYB, NAC and salt tolerance mechanisms particularly the relationship between ROS and antioxidant defenses (Tang, *et al.*, 2019). These studies demonstrate the significance of genetic approaches in

enhancing cotton resilience and advancing environmentally friendly Farming techniques.

4. Sufficient and Appropriate Fertilization

It has been shown that ensuring plants receive enough potassium (K) nutrition is essential for reducing the impacts of drought stress. Potassium is an important macronutrient that affects several processes necessary for metabolism and growth as well as cotton's water balance (Wang *et al.*, 2013). Research has demonstrated that, in comparison to untreated circumstances under stress, potassium fertilization significantly increases cotton production and its contributing components. Potassium (K) deficit could get worse the production of ROS in crops under drought stress (Cakmak *et al.*, 2005). K-deficient plants have significantly reduced CO₂ fixation because of impaired stomatal regulation during drought. However, the suppression of photosynthesis under moisture stress can be lessened by boosting extra-chloroplastic K⁺ concentrations with sufficient K⁺ administration (Egilla *et al.*, 2005). Its function in improving photosynthetic CO₂ fixation and the effective transport of photosynthates to sink tissues is associated with the increased K requirement in drought-exposed plants. By reducing the conversion of photosynthetic electrons to O₂, this process lowers the formation of ROS. Recent studies have shown that K fertilizer improves biomass output, downstream glucose metabolism, and leaf photosynthetic efficiency, increasing cotton's resilience to drought (Egilla *et al.*, 2005). It also helps with osmotic adjustment, water uptake, and reducing oxidative damage from ROS, underscoring the crucial function that K nutrition plays in plants' ability to withstand drought stress (Fig. 4)

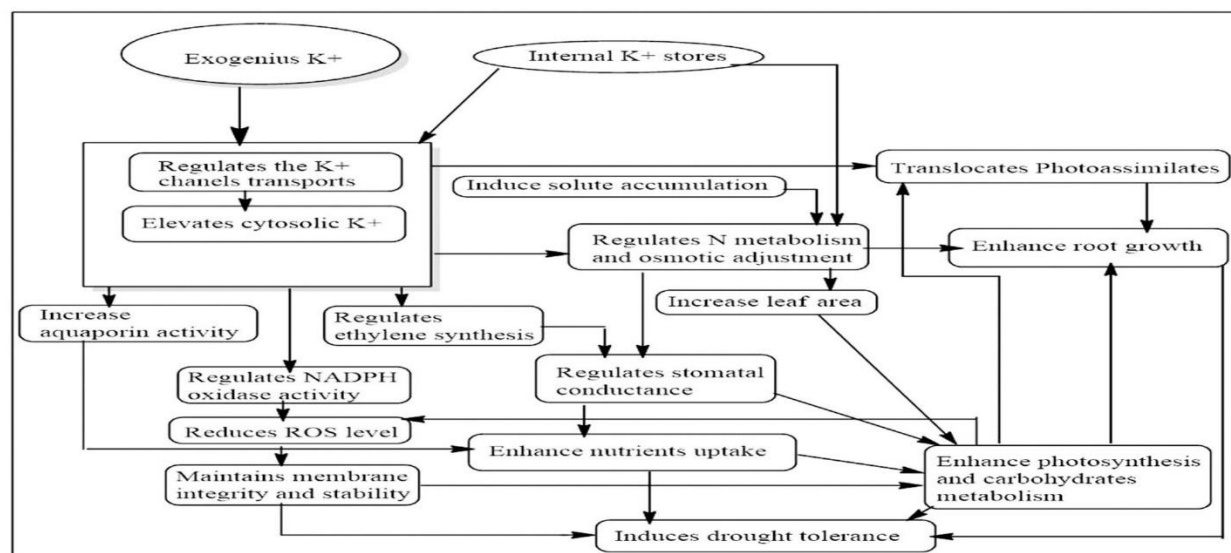


Figure 4: A potassium supplies improves photosynthesis, water use efficiency and root growth which increases cotton's resistance to drought. It helps plants withstand water deficiency conditions by promoting osmotic adjustment and cell integrity (Khan *et al.*, 2018).

5 Growth Regulating Agents

Plant growth regulators, both synthetic and natural, can mitigate the negative impacts of environmental stress on plant growth. Osmoprotectants and growth regulators used topically have been shown to increase cotton's resilience to drought. It has been observed that the application of hormones like abscisic acid, proline, gibberellic acid and salicylic acid improves resistance to moisture stress. These compounds protect cell structures and molecules under stress by increasing antioxidant activity, adjusting osmotic pressure and reducing ROS damage. Moreover, proline, betain and glycine boost stomatal conductance, transpiration and photosynthesis in cotton (Lichtfouse, *et al.*, 2009).

5.1 Jasmonic acid (JA)

The plant hormone jasmonic acid (JA) is crucial for helping plants cope with both stressors (Tan, *et al.*, 2012). It is involved in processes such as tendril coiling, fruit ripening, pollen production and root system development. When applied externally, jasmonates have been shown to enhance plant performance under dry conditions (Bandursk, *et al.*, 2003) and control stomatal dynamics (Riemann, *et al.*, 2015). The JAZ repressor proteins are essential to the well-understood jasmonic acid pathways and its production. In

the absence of JA, JAZ proteins bind with transcription factors diminish their activity. JA, on the other hand degrades JAZ proteins under water stress which enables to activate genes that confer tolerance to stress (Chini, *et al.*, 2007). Plant hormones frequently work in interrelated pathways, intricately coordinating a range of developmental and environmental responses (Riemann, *et al.*, 2015),

5.2 The Function of Gibberellic Acids (GA) in salt Stress Growth and Hormone

5.2.1 Biosynthesis of GA

Different stages of plant development depend on gibberellic acids, a class of plant hormones. They facilitate maturation and development of boll fibers, flowering and processes like root growth, leaf elongation and seed germination (Shi, *et al.*, 2019). There are about 130 different forms of gibberellin, although only a handful of them are biologically active (Finkelstein, *et al.*, 2008; Oleszewski, *et al.*, 2002). Later stages of their manufacturing and degradation control the generation of bioactive GAs (Zhang, *et al.*, 2014). GA 20-oxidase initiates the common biosynthetic pathway by converting precursor molecules into GA15, GA24, GA12 and GA9 (Figure 5). GA-3 oxidase then converts GA9 into GA4 (Reinecke, *et al.*, 2014; Qin, *et al.*, 2011).

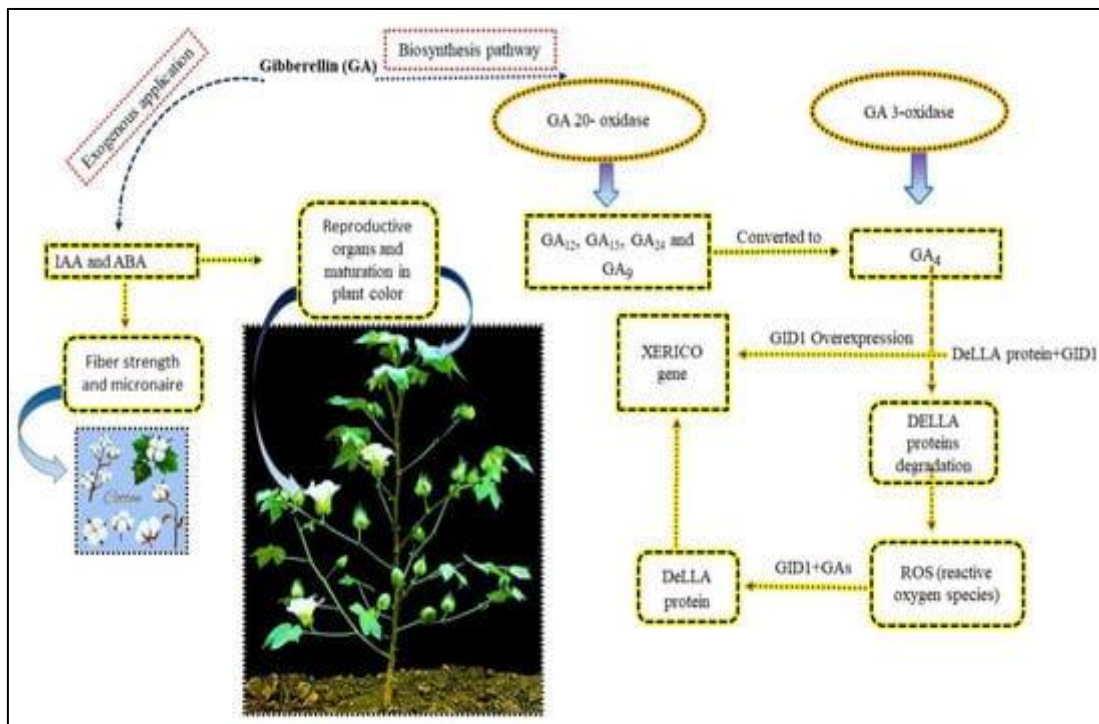


Figure 5: The biosynthesis route of exogenous gibberellic acid (GA) is essential for reducing salt stress in plants (Ahmad *et al.*, 2022)

Figure 5 The biosynthesis route of exogenous gibberellic acid (GA) is essential for reducing salt stress in plants. GA₂₄, GA₉, GA₁₅ and GA₁₂ are all converted by GA 20-oxidase and GA₉ is then further transformed into GA₄ by GA-3 oxidase. When DELLA proteins bind with GID1, the GA signaling pathway is triggered which causes DELLA proteins to degrade. Its breakdown under salt stress reduces the accumulation of ROS which in turn stimulates plant development. Plant sensitivity is increased when GID1 is overexpressed and when GID1 and GAS work together they assist send the GA signal to DELLA proteins, which control the expression of genes involved in GA production such as XERICO. These genes enhance plant growth and productivity while reducing the effects of salt stress. Additionally, exogenous GA increases the levels of ABA and IAA, which supports the growth and maturity of reproductive organs increases fiber strength and improves microneedle readings (Ahmad, *et al.*, 2022).

Based on research, gibberellic acids (GAs) are important for a number of biological activities that support crops vegetative and

reproductive growth while they are under stress. Exogenous GA treatments have been demonstrated to enhance the growth and fiber formation of cotton plants (Qin, *et al.*, 2011). Additionally, during growth of fiber GA treatment boosts the levels of abscisic acid and IAA which improves the natural color and fiber strength. The function of GA in cotton fiber growth and its connection to IAA and ABA levels under salt stress conditions need more investigation (Shu, *et al.*, 2018).

5.2.2 Transduction of GA Signaling

GA-insensitive dwarf1 (GID1) and DELLA proteins interact to trigger the activation of gibberellic acid (GA) signaling pathways. Particularly in situations of salt stress DELLA proteins are essential for coordinating different plant hormone signaling pathways and function as important negative regulators of GA. GID1 binds to bioactive GAs as a receptor and the GID1-GA complex helps the GA signal reach DELLA proteins which affects the expression of genes involved in GA production. It has been demonstrated that by lowering the buildup of ROS, the breakdown of DELLA proteins enhances plant viability under stress. However, plants may become

more sensitive to GAs if *GID1* is overexpressed. Furthermore, the *XERICO* gene which controls how plants react to salt stress interacts with DELLA proteins. By preventing GA production, DELLA proteins promote salt stress tolerance through the activation of *XERICO*. According to earlier research, *Arabidopsis thaliana*'s GA levels fall under salt stress, whereas DELLA protein accumulation increases (Yang, et al., 2008).

5.3 The Role of Salicylic Acid (SA) in Cotton Development and Growth of Plant and Under Salt Stress

5.3.1. Biosynthesis of SA

Chorismate is the starting point for the biosynthesis of SA which is accomplished by a number of metabolic processes including the ICS and PAL pathways. Both the cytosol and the chloroplast are sites of this biosynthesis (Dempsey and Klessig, 2017). Several environmental stressors have an impact on the enzymes involved in this process, which are essential for controlling SA function (Khan, et al., 2015). The production of SA and

plant responses to pathogen-induced illnesses are significantly influenced by the ICS pathway in particular. In *Arabidopsis*, the ICS pathway plays a key role in the accumulation of salicylic acid (SA), while in rice, the PAL pathway has been identified as more significant for SA production (Lefevere, et al., 2020). More research is necessary to determine whether these processes could lead to increased SA buildup in cotton. Research has demonstrated that various plant tissues may regulate SA biosynthesis differently. For example, in rice the concentration of SA in the shoots is notably greater than in roots (Duan, et al., 2014). By triggering the action of protein kinases like cAMP and cGMP, SA has been connected to the control closure of stomata (Hao, et al., 2010). As secondary messengers, cGMP and cAMP both control a number of physiological functions in plants including cell cycle and gene expression maintenance. The entire spectrum of metabolic processes that SA regulates is still unknown (Figure 6).

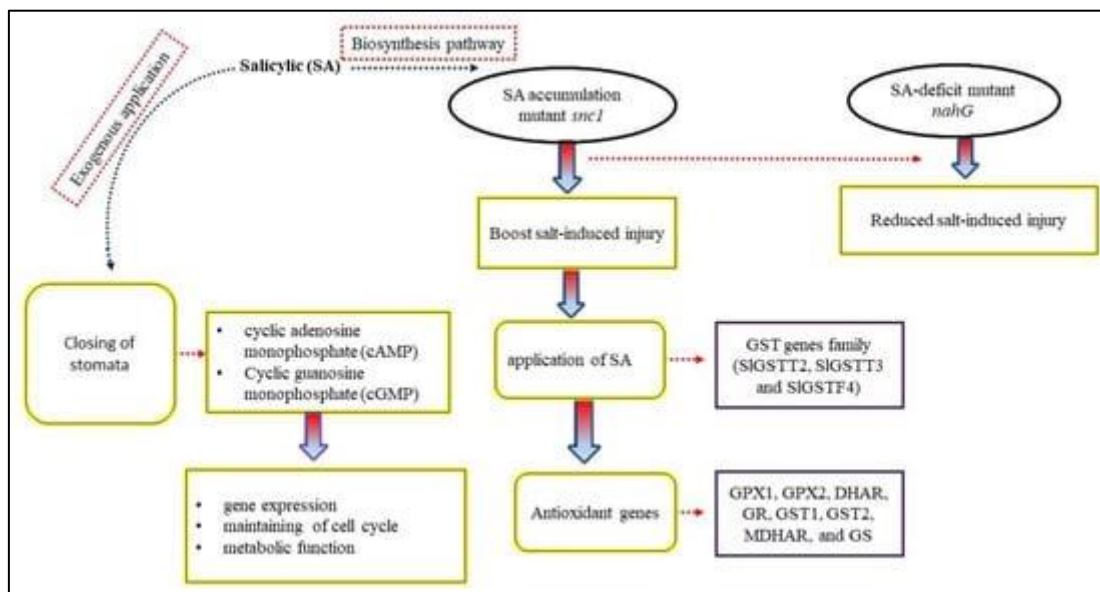


Figure 6: By altering its biosynthesis pathway, an external supply of salicylic acid (SA) is essential for reducing salt stress (Ahmad et al., 2022)

Figure 6 By altering its biosynthesis pathway, an external supply of salicylic acid (SA) is essential for reducing salt stress. Salt-induced injury occurs in mutants such as *snc1* which accumulate larger levels of SA, while the SA-deficient mutant lessens salt stress by inhibiting SA signaling specifically *npr1-1* and

snc1. By modifying the expression of genes from the GST family, including *SIGSTT4*, *SIGSTT3* and *SIGSTT2* as well as antioxidant genes including, *GST1*, *GPX1*, *DHAR*, *MDHAR* and *GR*. SA application helps reduce salt-induced damage. Additionally, SA controls stomatal closure during salt stress by

means of secondary messengers such as cAMP and cGMP, which in turn control a range of gene expression, physiological processes, the upkeep of cell cycles and metabolic processes in plants (Ahmad, *et al.*, 2022).

5.3.2. SA Signaling Transduction

In plants, SA is an essential signaling compound that regulates responses to a variety of abiotic stresses, including salinity, ozone, heavy metals and heat (Shah, *et al.*, 2003). Two major signaling pathways, PAL and ICS are involved in the biosynthesis of SA in plants (Zhao, *et al.*, 2017). In tobacco, PAL genes are silenced and in Arabidopsis, SA accumulation is decreased when PAL activity is inhibited (Wildermuth, *et al.*, 2001). Through the action of ICS in the ICS pathway, SA is produced in Arabidopsis from chorismate via isochorismate (Dempsey, *et al.*, 2011). To increase crop resilience more research is required to examine SA buildup in cotton under salinity stress. In order to increase crop yield SA is also transformed into a variety of forms including glucosylation, methylation, hydroxylation and transferred to other plant sections (Pieterse, *et al.*, 2012). Additionally, a complex signaling network that controls plant development and stress responses is formed by the interaction of SA with other hormones such, abscisic acid, jasmonic acid, auxin and ethylene.

6 The role of miRNAs in Reducing Drought Stress

Plants need the help of tiny, non-coding RNA molecules called microRNAs (miRNAs) to respond to a range of abiotic stresses (Ding, *et al.*, 2017). Through hormonal signaling pathways such SA, ABA and ET which are implicated in desiccation tolerance, these miRNAs control plant responses (Huang, *et al.*, 2008). MiRNAs unique to drought tolerance have been found in crops such as *Brassica napus* and cotton (Guo, *et al.*, 2016). By influencing gene expression, these miRNAs aid plants in adjusting to challenging environmental circumstances (Fig. 5). Since extended water stress can result in metabolic disturbances and nutritional imbalances in plants, improving cotton's fiber development and drought resistance is a major area of focus in agriculture (Dong, *et al.*, 2015). It has been demonstrated that overexpressing genes such GhCIPK6 and GhMPK2 increases cotton's resistance to drought suggesting that these genes may be genetically improved (He, *et al.*, 2013; Zhang, *et al.*, 2011). Numerous miRNAs, including, miR396, miR172, miR164 and miR6158 have been found in studies to target genes that are used to produce cotton fiber. It has been discovered that 163 miRNAs target 210 genes linked to fiber production. Although further research is needed to fully understand how these miRNAs contribute to stress tolerance, they offer a promising strategy for making cotton more resilient to severe weather.

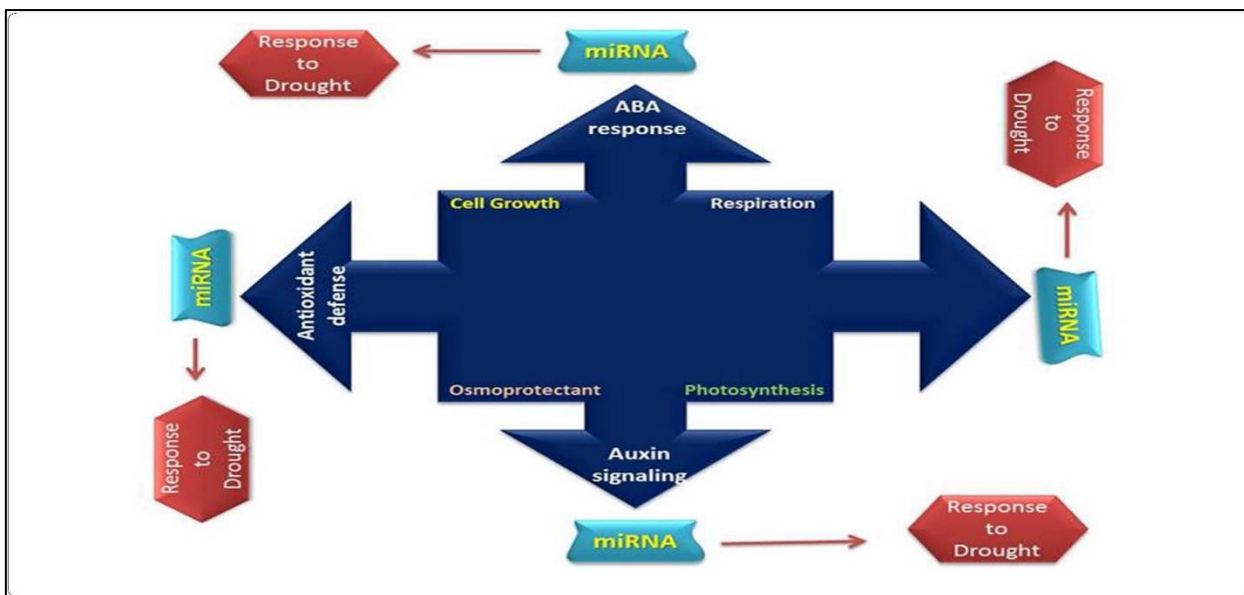


Fig. 7 MiRNAs and many cellular mechanisms work together to help plants withstand drought (Khan, et al., 2018)

7. Future Opportunities and Difficulties

7.1 Cutting-Edge Biotechnological Strategies to Reduce Abiotic Stress

Crop yields have been successfully raised by traditional breeding methods but it has proven more difficult to create crops that are resistant to abiotic stressors. Breeding methods frequently concentrate on assessing genetic material under optimal conditions ignoring the complications that many stress factors bring during the plant's life cycle. This contributes to the difficulty. Because of this, traditional breeding finds it difficult to choose plants that are more resilient to these challenges underscoring the need for alternate techniques to raise agricultural output and quality in these circumstances. By changing gene expression using a variety of transformation procedures and tissue culture approaches genetic manipulation through transgenic plants has showed promise in recent years (Guo, et al., 2020). By altering the expression of specific genes, researchers have gained a better understanding of the mechanisms underlying plant stress responses (Abdulrahemm, et al., 2024). Engineering developments and new genome-wide approaches are offering vital resources for investigating how epigenetic modifications may affect plants' capacity to

endure the stress of the surroundings (Choudhary, et al., 2023). Crop resilience can be improved by epigenetic modification in response to stress (Dos Santos, et al., 2023). In order to produce stress-resistant plant varieties, transgenic techniques are concentrating on improving mechanisms such osmolyte synthesis, antioxidant defenses, signal transduction and gene modulation (Li, et al., 2022). Crop biotechnology has exciting prospects thanks to new technologies like CRISPR gene editing tools (Ndudzo, et al., 2024). Because of the accuracy of CRISPR/Cas technology small DNA alterations can be made without introducing significant foreign DNA sequences increasing the likelihood that genetically modified crops will be approved around the world.

7.2 Cotton's Difficulties in Reducing Abiotic Stress

Conventional breeding methods have historically been used to meet the increasing needs brought on by population increase and climate change by transferring advantageous genes into superior cultivars. However, more effective solutions have been found with more recent genetic engineering (GE) tools including ZFNs, TALENs and CRISPR/Cas. Of them, the CRISPR/Cas system is highly regarded due of its simplicity, effectiveness and adaptability (Gaj, et al., 2016). In order to

resolve regulatory concerns around genetically modified organisms (GMOs), researchers are using CRISPR/Cas technology to boost cotton's resistance to both stressors, alter gene expression and stack advantageous features without adding foreign genes (Nduzo, et al., 2024).

In order to increase the economic value of cotton farming, this approach offers chances to improve cotton fiber quality, plant structure, flowering and address problems with epigenetic modifications and gene stacking. Numerous biotic and abiotic factors including salt, drought, intense temperatures and heavy metal toxicity have a substantial impact on cotton agriculture and have an effect on plant growth and fiber quality. Modern farming methods and molecular techniques must be combined in breeding projects to improve cotton's stress tolerance in order to address these problems. To better understand cotton's stress reactions and increase its resilience, a multidisciplinary approach combining physiological, biochemical, and molecular studies is essential, guaranteeing more sustainable farming methods.

8. Conclusion

This paper offers a thorough analysis of the molecular, biochemical and physiological responses of cotton to abiotic stressors. Cotton's exceptional resilience and capacity to trigger a variety of defensive mechanisms to lessen the negative impacts of these stresses are highlighted through a thorough analysis. Discovering the intricate molecular mechanisms underlying abiotic stress tolerance in plants requires the use of contemporary genomic technologies, including quantitative trait locus, mapping, high-throughput sequencing, marker-assisted selection RNA sequencing, single-cell sequencing, chromatin immunoprecipitation sequencing, genome-wide association studies (GWAS), metabolomics, proteomics, genomic selection and multi-omics approaches, as well as CRISPR-based genome editing techniques. The development of transgenic cotton lines that carry several resistance genes and

provide improved tolerance to a variety of abiotic stressors is currently the main emphasis. In this sense, the use of gene-editing technologies, especially CRISPR/Cas9 has enormous potential for producing cotton varieties with stacked resistance traits, which would increase their ability to adapt to changing environmental conditions and promote climate change-resilient agricultural practices.

References

1. Ahmad, I., Zhu, G., Zhou, G., Song, X., Ibrahim, M. E. H., Salih, E. G. I., Hussain, S. & Younas, M. U. "Pivotal role of phytohormones and their responsive genes in plant growth and their signaling and transduction pathway under salt stress in cotton." *International Journal of Molecular Sciences*, 23.13 (2022): 7339.
2. Egilla, J. N., Davies, F. T. & Boutton, T. W. "Drought stress influences leaf water content, photosynthesis, and water-use efficiency of *Hibiscus rosa-sinensis* at three potassium concentrations." *Photosynthetica*, 43 (2005): 135-140.
3. Bandurska, H., Stroiński, A. & Kubiś, J. "The effect of jasmonic acid on the accumulation of ABA, proline, and spermidine and its influence on membrane injury under water deficit in two barley genotypes." *Acta Physiologiae Plantarum*, 25.3 (2003): 279-285.
4. Riemann, M., Dhakarey, R., Hazman, M., Miro, B., Kohli, A. & Nick, P. "Exploring jasmonates in the hormonal network of drought and salinity responses." *Frontiers in Plant Science*, 6 (2015): 1077.
5. Ahmad, P., Rasool, S., Gul, A., Akram, N. A., Ashraf, M. & Gucel, S. "Jasmonates: multifunctional roles in stress tolerance." *Frontiers in Plant Science*, 7 (2016): 813.
6. Abdullah, M., Ahmad, F., Zang, Y., Jin, S., Ahmed, S., Li, J., Islam, F., Ahmad, M., Zhang, Y. & Hu, Y. "HEAT-RESPONSIVE PROTEIN regulates heat stress via fine-tuning ethylene/auxin signaling pathways in cotton." *Plant Physiology*, 191 (2022): 772-788.
7. Abdulraheem, M. I., Xiong, Y., Moshood, A. Y., Cadenas-Pliego, G., Zhang, H. &

- Hu, J. "Mechanisms of plant epigenetic regulation in response to plant stress: recent discoveries and implications." *Plants*, 13 (2024): 163.
8. Abid, M. A., Liang, C., Malik, W., Meng, Z., Tao, Z., Meng, Z., Ashraf, J., Guo, S. & Zhang, R. "Cascades of ionic and molecular networks involved in expression of genes underpin salinity tolerance in cotton." *Journal of Plant Growth Regulation*, 37 (2018): 668-679.
 9. Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van Der Straeten, D., Peng, J. & Harberd, N. P. "Integration of plant responses to environmentally activated phytohormonal signals." *Science*, 311 (2006): 91-94.
 10. Anjum, S. A., Wang, C., Farooq, M., Hussain, M. & Xue, L. "Brassinolide application improves drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange." *Journal of Agronomy and Crop Science*, 197 (2011): 177-185.
 11. Baillo, E. H., Kimotho, R. N., Zhang, Z. & Xu, P. "Transcription factors associated with abiotic and biotic stress tolerance and their potential for crop improvement." *Genes*, 10 (2019): 771.
 12. Banerjee, A. & Roychoudhury, A. "WRKY proteins: Signaling and regulation of expression during abiotic stress responses." *The Scientific World Journal*, 2015 (2015): 807560.
 13. Basso, M. F., Costa, J. A., Ribeiro, T. P., Arraes, F. B. M., Lourenço-Tessutti, I. T., Macedo, A. F., Neves, M. R. D., Nardeli, S. M., Arge, L. W., Perez, C. E. A, et al. "Overexpression of the *CaHB12* transcription factor in cotton (*Gossypium hirsutum*) improves drought tolerance." *Plant Physiology and Biochemistry*, 165 (2021): 80-93.
 14. Bitá, C. & Gerats, T. "Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress-tolerant crops." *Frontiers in Plant Science*, 4 (2013): 273.
 15. Cakmak, I. "The role of potassium in alleviating detrimental effects of abiotic stresses in plants." *Journal of Plant Nutrition and Soil Science*, 168 (2005): 521-530.
 16. Chen, L., Zhang, B., Xia, L., Yue, D., Han, B., Sun, W., Wang, F., Lindsey, K., Zhang, X. & Yang, X. "The GhMAP3K62-GhMKK16-GhMPK32 kinase cascade regulates drought tolerance by activating GhEDT1-mediated ABA accumulation in cotton." *Journal of Advanced Research*, 51 (2023): 13-25.
 17. Chini, A. "The JAZ family of repressors is the missing link in jasmonate signaling." *Nature*, 448 (2007): 666-671.
 18. Choudhary, S., Wani, K. I., Naeem, M., Khan, M. M. A. & Aftab, T. "Cellular responses, osmotic adjustments, and role of osmolytes in providing salt stress resilience in higher plants: Polyamines and nitric oxide crosstalk." *Journal of Plant Growth Regulation* (2023). (Incomplete reference – please provide volume and page numbers for correction)
 19. "CRISPR/Cas system for generation of pathogen-resistant plants." *Biochemistry (Moscow)*, 83 (2018): 1552-1562.
 20. Dempsey, D. & Klessig, D. F. "How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans?" *BMC Biology*, 15 (2017): 23.
 21. Dempsey, D. M. A., Vlot, A. C., Wildermuth, M. C. & Klessig, D. F. "Salicylic acid biosynthesis and metabolism." *The Arabidopsis Book/American Society of Plant Biologists*, 9 (2011): e0156.
 22. Ding, Y., Ma, Y., Liu, N., Xu, J., Hu, Q., Li, Y., Wu, Y., Xie, S., Zhu, L., Min, L. & Zhang, X. L. "MicroRNAs involved in auxin signaling modulate male sterility under high-temperature stress in cotton (*Gossypium hirsutum*)." *The Plant Journal*, 91 (2017): 977-992.
 23. Dong, T., Park, Y. & Hwang, I. "Abscisic acid: Biosynthesis, inactivation, homeostasis, and signaling." *Essays in Biochemistry*, 58 (2015): 29-48.
 24. Dos Santos, C. & Franco, O. L. "Pathogenesis-related proteins (PRs) with enzyme activity activating plant defense responses." *Plants*, 12 (2023): 2226.
 25. Duan, L., Liu, H., Li, X., Xiao, J. & Wang, S. "Multiple phytohormones and phytoalexins are involved in disease resistance to

- Magnaporthe oryzae* invaded from roots in rice." *Physiologia Plantarum*, 152 (2014): 486-500.
26. Finkelstein, R., Reeves, W., Ariizumi, T. & Steber, C. "Molecular aspects of seed dormancy." *Annual Review of Plant Biology*, 59 (2008): 387-415.
 27. Gaj, T., Gersbach, C. A. & Barbas, C. F. "ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering." *Trends in Biotechnology*, 31 (2013): 397-405.
 28. Gaj, T., Sirk, S. J., Shui, S. L. & Liu, J. "Genome-editing technologies: Principles and applications." *Cold Spring Harbor Perspectives in Biology*, 8 (2016): a023754.
 29. Guo, M., Liu, J. H., Ma, X., Luo, D. X., Gong, Z. H. & Lu, M. H. "The plant heat stress transcription factors (HSFs): Structure, regulation, and function in response to abiotic stresses." *Frontiers in Plant Science*, 7 (2016): 114.
 30. Guo, Q., Meng, S., Tao, S., Feng, J., Fan, X., Xu, P., Xu, Z. & Shen, X. "Overexpression of a samphire high-affinity potassium transporter gene *SbHKT1* enhances salt tolerance in transgenic cotton." *Acta Physiologiae Plantarum*, 42 (2020): 36.
 31. Guo, Y., Chen, Q., Qu, Y., Deng, X., Zheng, K., Wang, N., Shi, J., Zhang, Y., Chen, Q. & Yan, G. "Development and identification of molecular markers of *GhHSP70-26* related to heat tolerance in cotton." *Gene*, 874 (2023): 147486.
 32. Han, W., Zhao, J., Deng, X., Gu, A., Li, D., Wang, Y., Lu, X., Zu, Q., Chen, Q., Chen, Q., et al. "Quantitative trait locus mapping and identification of candidate genes for resistance to *Fusarium* wilt race 7 using a resequencing-based high-density genetic bin map in a recombinant inbred line population of *Gossypium barbadense*." *Frontiers in Plant Science*, 13 (2022): 815643.
 33. Hao, F., Zhao, S., Dong, H., Zhang, H., Sun, L. & Miao, C. "*Nia1* and *Nia2* are involved in exogenous salicylic acid-induced nitric oxide generation and stomatal closure in *Arabidopsis*." *Journal of Integrative Plant Biology*, 52 (2010): 298-307.
 34. He, L., Yang, X., Wang, L., Zhu, L., Zhou, T., Deng, J. & Zhang, X. "Molecular cloning and functional characterization of abiotic stress tolerance in transgenic plants." *Biochemical and Biophysical Research Communications*, 435 (2013): 209-215.
 35. He, Z., Chen, M., Ling, B., Cao, T., Wang, C., Li, W., Tang, W., Chen, K., Zhou, Y., Chen, J., et al. "Overexpression of the autophagy-related gene *SiATG8a* from foxtail millet (*Setaria italica* L.) in transgenic wheat confers tolerance to phosphorus starvation." *Plant Physiology and Biochemistry*, 196 (2023): 580-586.
 36. Huang, Q. S., Wang, H. Y., Gao, P., Wang, G. Y. & Xia, G. X. "Cloning and characterization of a calcium-dependent protein kinase gene associated with cotton fiber development." *Plant Cell Reports*, 27 (2008): 1869-1875.
 37. Ijaz, A., Anwar, Z., Ali, A., Ditta, A., Shani, M. Y., Haidar, S. & Khan, M. K. R. "Unraveling the genetic and molecular basis of heat stress in cotton." *Frontiers in Genetics*, 15 (2024): 1296622.
 38. Jabran, K., Ul-Allah, S. & Chauhan, B. S. "An introduction to global production trends and uses, history and evolution, and genetic and biotechnological improvements in cotton." In: *Cotton Production*, edited by John Wiley & Sons Ltd., Hoboken, NJ, USA, 2020, Vol. 18, pp. 1-22.
 39. Javaid, D., Ganie, S. Y., Hajam, Y. A. & Reshi, M. S. "CRISPR/Cas9 system: A reliable and facile genome editing tool in modern biology." *Molecular Biology Reports*, 49 (2022): 12133-12150.
 40. Ju, F., Pang, J., Sun, L., Gu, J., Wang, Z., Wu, X., Ali, S., Wang, Y., Zhao, W., Wang, S., et al. "Integrative transcriptomic, metabolomic, and physiological analyses revealed the physiological and molecular mechanisms by which potassium regulates the salt tolerance of cotton (*Gossypium hirsutum* L.) roots." *Industrial Crops and Products*, 193 (2023): 116177.
 41. Khan, A., Pan, X., Najeeb, U., Tan, D. K. Y., Fahad, S., Zahoor, R. & Luo, H. "Coping with drought: Stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness." *Biological Research*, 51 (2018).

42. Khan, M. I. R., Fatma, M., Per, T. S., Anjum, N. A. & Khan, N. A. "Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants." *Frontiers in Plant Science*, 6 (2015): 462.
43. Koo, Y. M., Heo, A. Y. & Choi, H. W. "Salicylic acid as a safe plant protector and growth regulator." *The Plant Pathology Journal*, 36 (2020): 1.
44. Kopecka, R., Kameniarova, M., Cerny, M., Brzobohaty, B. & Novak, J. "Abiotic stress in crop production." *International Journal of Molecular Sciences*, 24 (2023): 6603.
45. Kushanov, F. N., Turaev, O. S., Ernazarova, D. K., Gapparov, B. M., Oripova, B. B., Kudratova, M. K., Rafieva, F. U., Khalikov, K. K., Erjigitov, D. S., Khidirov, M. T, et al. "Genetic diversity, QTL mapping, and marker-assisted selection technology in cotton (*Gossypium* spp.)." *Frontiers in Plant Science*, 12 (2021): 779386.
46. Lefevre, H., Bauters, L. & Gheysen, G. "Salicylic acid biosynthesis in plants." *Frontiers in Plant Science*, 11 (2020): 338.
47. Li, C., Unver, T. & Zhang, B. "A high-efficiency CRISPR/Cas9 system for targeted mutagenesis in cotton (*Gossypium hirsutum* L.)." *Scientific Reports*, 7 (2017): 43902.
48. Li, C., Xu, M., Cai, X., Han, Z., Si, J. & Chen, D. "Jasmonate signaling pathway modulates plant defense, growth, and their trade-offs." *International Journal of Molecular Sciences*, 23 (2022): 3945.
49. Li, L. B., Yu, D. W., Zhao, F. L., Pang, C. Y., Song, M. Z., Wei, H. L., Fan, S. L. & Yu, S. X. "Genome-wide analysis of the calcium-dependent protein kinase gene family in *Gossypium raimondii*." *Journal of Integrative Agriculture*, 14 (2015): 29-41.
50. Li, Y., Hu, Z., Dong, Y. & Xie, Z. "Overexpression of the cotton trihelix transcription factor GhGT23 in *Arabidopsis* mediates salt and drought stress tolerance by binding to GT and MYB promoter elements in stress-related genes." *Frontiers in Plant Science*, 14 (2023): 1144650.
51. Lichtfouse, E., Navarrete, M., Debaeke, P., Souchère, V., Alberola, C. & Ménassieu, J. "Agronomy for sustainable agriculture: A review." *Agronomy for Sustainable Development*, 29 (2009): 1-6.
52. Lisei-De-Sá, M. E., Arraes, F. B. M., Brito, G. G., Beneventi, M. A., Lourenço-Tessutti, I. T., Basso, A. M. M., Amorim, R. M. S., Silva, M. C. M., Faheem, M., Oliveira, N. G, et al. "AtDREB2A-CA influences root architecture and increases drought tolerance in transgenic cotton." *Agricultural Sciences*, 8 (2017): 1195-1225.
53. Liu, R., Shen, Y., Wang, M., Liu, R., Cui, Z., Li, P., Wu, Q., Shen, Q., Chen, J., Zhang, S, et al. "GhMYB102 promotes drought resistance by regulating drought-responsive genes and ABA biosynthesis in cotton (*Gossypium hirsutum* L.)." *Plant Science*, 329 (2023): 111608.
54. Liu, Z., Ge, X., Yang, Z., Zhang, C., Zhao, G., Chen, E., Liu, J., Zhang, X. & Li, F. "Genome-wide identification and characterization of SnRK2 gene family in cotton (*Gossypium hirsutum* L.)." *BMC Genetics*, 18 (2017): 54.
55. Lv, S., Zhang, K., Gao, Q., Lian, L., Song, Y. & Zhang, J. "Overexpression of an H⁺-PPase gene from *Thellungiella halophila* in cotton enhances salt tolerance and improves growth and photosynthetic performance." *Plant Cell Physiology*, 49 (2008): 1150-1164.
56. Makarova, S. S., Khromov, A. V., Spechenkova, N. A., Taliansky, M. E. & Kalinina, N. O. "Application of genome-editing technologies in plant biotechnology." (*Incomplete reference – please provide details for correction*)
57. Maqbool, A., Abbas, W., Rao, A. Q., Hussain, T., Iqbal, M. M., Shahid, A. A., Husnain, T. & Riazuddin, S. "*Gossypium arboreum* GHSP26 enhances drought tolerance in *Gossypium hirsutum*." *Biotechnology Progress*, 26 (2009): 21-25.
58. Miladinovic, D., Antunes, D., Yildirim, K., Bakhsh, A., Cvejić, S., Kondić-Špika, A., Jeromela, A. M., Opsahl-Sorteberg, H. G., Zambounis, A. & Hilioti, Z. "Targeted plant improvement through genome editing: From laboratory to field." *Plant Cell Reports*, 40 (2021): 935-951.
59. Misra, A., Singh, P. K. & Pandey, S. N. "Improvement of cotton varieties using

- biotechnological tools: A review." *Journal of Applied Biosciences*, 50 (2024): 24-29.
60. Mwando, E., Han, Y., Angessa, T. T., Zhou, G., Hill, C. B., Zhang, X. Q. & Li, C. "Genome-wide association study of salinity tolerance during germination in barley (*Hordeum vulgare* L.)." *Frontiers in Plant Science*, 11 (2020): 118.
 61. Ndudzo, A., Makuvise, A. S., Moyo, S. & Bobo, E. D. "CRISPR-Cas9 genome editing in crop breeding for climate change resilience: Implications for smallholder farmers in Africa." *Journal of Agriculture and Food Research*, 16 (2024): 101132.
 62. Noreen, S., Ashraf, M., Hussain, M., Perveen, S. & Ahmad, R. "Abiotic stresses mediated changes in morphophysiology of cotton plants." In: *Cotton Production and Uses*, edited by S. Ahmad & M. Hasanuz-zaman, Springer: Singapore, 2020, pp. 377-393.
 63. Olszewski, N., Sun, T. P. & Gubler, F. "Gibberellin signaling: Biosynthesis, catabolism, and response pathways." *The Plant Cell*, 14 (2002): S61-S80.
 64. Oluoch, G., Zheng, J., Wang, X., Khan, M. K. R., Zhou, Z., Cai, X., Wang, C., Wang, Y., Li, X., Wang, H, et al. "QTL mapping for salt tolerance at seedling stage in the interspecific cross of *Gossypium tomentosum* with *Gossypium hirsutum*." *Euphytica*, 209 (2016): 223-235.
 65. Patil, A. M., Pawar, B. D., Wagh, S. G., Markad, N. R., Bhute, N. K., Shinde, H., Shelake, R. M., Červený, J. & Wagh, R. S. "Unravelling abiotic stress physiology in cotton (*Gossypium hirsutum* L.): Biotechnological interventions in mitigating abiotic stress." *Agriculture*, 14.9 (2024): 1638.
 66. Patil, A. M., Pawar, B. D., Wagh, S. G., Shinde, H., Shelake, R. M., Markad, N. R., Bhute, N. K., Červený, J. & Wagh, R. S. "Abiotic stress in cotton: Insights into plant responses and biotechnological solutions." *Agriculture*, 14.9 (2024): 1638.
 67. Pérez-Clemente, R. M., Vives, V., Zandalinas, S. I., López-Climent, M. F., Muñoz, V. & Gómez-Cadenas, A. "Biotechnological approaches to study plant responses to stress." *BioMed Research International*, 2013 (2013): 654120.
 68. Pieterse, C. M., Van der Does, D., Zamioudis, C., Leon-Reyes, A. & Van Wees, S. C. "Hormonal modulation of plant immunity." *Annual Review of Cell and Developmental Biology*, 28 (2012): 489-521.
 69. Pilon, C., Snider, J. L., Sobolev, V., Chastain, D. R., Sorensen, R. B., Meeks, C. D., Massa, A. N., Walk, T., Singh, B. & Earl, H. J. "Assessing stomatal and non-stomatal limitations to carbon assimilation under progressive drought in peanut (*Arachis hypogaea* L.)." *Journal of Plant Physiology*, 231 (2018): 124-134.
 70. Qi, Q., Wang, N., Ruan, S., Muhammad, N., Zhang, H., Shi, J., Dong, Q., Xu, Q., Song, M., Yan, G, et al. "Mepiquat chloride priming confers the ability of cotton seed to tolerate salt by promoting ABA-operated GABA signaling control of the ascorbate-glutathione cycle." *Journal of Cotton Research*, 6 (2023): 24.
 71. Qin, F., Kodaira, K.-S., Maruyama, K., Mizoi, J., Tran, L.-S. P., Fujita, Y., Morimoto, K., Shinozaki, K. & Yamaguchi-Shinozaki, K. "Spindly: A negative regulator of gibberellic acid signaling, is involved in the plant abiotic stress response." *Plant Physiology*, 157 (2011): 1900-1913.
 72. Reinecke, D. M., Wickramarathna, A. D., Ozga, J. A., Kurepin, L. V., Jin, A. L., Good, A. G. & Pharis, R. P. "Gibberellin 3-oxidase gene expression patterns influence gibberellin biosynthesis, growth, and development in pea." *Plant Physiology*, 163 (2013): 929-945.
 73. Said, J. I., Lin, Z., Zhang, X., Song, M. & Zhang, J. "A comprehensive meta-QTL analysis for fiber quality, yield, yield-related and morphological traits, drought tolerance, and disease resistance in tetraploid cotton." *BMC Genomics*, 14 (2013): 776.
 74. Schluttenhofer, C. & Yuan, L. "Regulation of specialized metabolism by WRKY transcription factors." *Plant Physiology*, 167 (2015): 295-306.
 75. Seleiman, M. F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Abdul-Wajid, H. H. & Battaglia, M. L. "Drought stress impacts on plants and different approaches to al-

- leviate its adverse effects." *Plants*, 10 (2021): 259.
76. Shah, J. "The salicylic acid loop in plant defense." *Current Opinion in Plant Biology*, 6 (2003): 365-371.
77. Shelake, R. M., Kadam, U. S., Kumar, R., Pramanik, D., Singh, A. K. & Kim, J. Y. "Engineering drought and salinity tolerance traits in crops through CRISPR-mediated genome editing: Targets, tools, challenges, and perspectives." *Plant Communications*, 3 (2022): 100417.
78. Shelake, R. M., Pramanik, D. & Kim, J.-Y. "CRISPR base editor-based targeted random mutagenesis (BE-TRM) toolbox for directed evolution." *BMB Reports*, 57 (2024): 30-39.
79. Shi, J. B., Wang, N., Zhou, H., Xu, Q. H. & Yan, G. T. "The role of gibberellin synthase gene *GhGA2ox1* in upland cotton (*Gossypium hirsutum* L.) responses to drought and salt stress." *Biotechnology and Applied Biochemistry*, 66 (2019): 298-308.
80. Shu, K., Zhou, W., Chen, F., Luo, X. & Yang, W. "Abscisic acid and gibberellins antagonistically mediate plant development and abiotic stress responses." *Frontiers in Plant Science*, 9 (2018): 416.
81. Shukla, R. P., Tiwari, G. J., Joshi, B., Song-Beng, K., Tamta, S., Boopathi, N. M. & Jena, S. N. "GBS-SNP and SSR-based genetic mapping and QTL analysis for drought tolerance in upland cotton." *Physiology and Molecular Biology of Plants*, 27 (2021): 1731-1745.
82. Snider, J. L., Collins, G. D., Whitaker, J., Perry, C. D. & Chastain, D. R. "Electron transport through photosystem II is not limited by a wide range of water deficit conditions in field-grown *Gossypium hirsutum*." *Journal of Agronomy and Crop Science*, 200 (2014): 77-82.
83. Sun, F., Yang, Y., Wang, P., Ma, J. & Du, X. "Quantitative trait loci and candidate genes for yield-related traits of upland cotton revealed by genome-wide association analysis under drought conditions." *BMC Genomics*, 24 (2023): 531.
84. Sun, R., Tian, R., Ma, D., Wang, S. & Liu, C. "Comparative transcriptome study provides insights into acquisition of embryogenic ability in upland cotton during somatic embryogenesis." *Journal of Cotton Research*, 1 (2018): 9.
85. Tan, J., Tu, L., Deng, F., Wu, R. & Zhang, X. "Exogenous jasmonic acid inhibits cotton fiber elongation." *Journal of Plant Growth Regulation*, 31 (2012): 599-605.
86. Tang, Y., Bao, X., Zhi, Y., Wu, Q., Guo, Y., Yin, X., Zeng, L., Li, J., Zhang, J., He, W, et al. "Overexpression of a MYB family gene, OsMYB6, increases drought and salinity stress tolerance in transgenic rice." *Frontiers in Plant Science*, 10 (2019): 168.
87. Tian, M., Wu, A., Zhang, M., Zhang, J., Wei, H., Yang, X., Ma, L., Lu, J., Fu, X., Wang, H, et al. "Genome-wide identification of the early flowering 4 (ELF4) gene family in cotton and silent GhELF4-1 and GhEFL3-6 decreased cotton stress resistance." *Frontiers in Genetics*, 12 (2021): 686852.
88. Tyler, L., Thomas, S. G., Hu, J., Dill, A., Alonso, J. M., Ecker, J. R. & Sun, T. P. "DELLA proteins and gibberellin-regulated seed germination and floral development in *Arabidopsis*." *Plant Physiology*, 135 (2004): 1008-1019.
89. Ueguchi-Tanaka, M., Nakajima, M., Motoyuki, A. & Matsuoka, M. "Gibberellin receptor and its role in gibberellin signaling in plants." *Annual Review of Plant Biology*, 58 (2007): 183-198.
90. Verma, A. K., Mandal, S., Tiwari, A., Monachesi, C., Catassi, G. N., Srivastava, A., Gatti, S., Lionetti, E. & Catassi, C. "Current status and perspectives on the application of CRISPR/Cas9 gene-editing system to develop a low-gluten, non-transgenic wheat variety." *Foods*, 10 (2021): 2351.
91. Wagh, S. G., Kobayashi, K., Yaeno, T., Yamaoka, N., Masuta, C. & Nishiguchi, M. "Rice necrosis mosaic virus, a fungal-transmitted *Bymovirus*: Complete nucleotide sequence of the genomic RNAs and subgrouping of bymoviruses." *Journal of General Plant Pathology*, 82 (2016): 38-42.
92. Wang, M., Zheng, Q., Shen, Q. & Guo, S. "The critical role of potassium in plant stress response." *International Journal of Molecular Sciences*, 14 (2013): 7370-7390.

93. Wang, K., Gao, Y., Li, S., Zhang, M., Wu, Z., Liu, L., Sun, H., Li, C. & Zhang, Y. "Response of leaf stomata and photosynthetic parameters to short-term drought stress in cotton (*Gossypium hirsutum* L.)." *Chinese Journal of Eco-Agriculture*, 27 (2019): 901-907.
94. Wang, K., Wang, Z., Li, F., Ye, W., Wang, J., Song, G., Yue, Z., Cong, L., Shang, H., Zhu, S, et al. "The draft genome of a diploid cotton *Gossypium raimondii*." *Nature Genetics*, 44 (2012): 1098-1103.
95. Wang, R., Liu, L., Kong, Z., Li, S., Lu, L., Kabir, N., Chen, G., Zhang, J., Qanmber, G. & Liu, Z. "Identification of stress-responsive genes in *Gossypium hirsutum* L." *Plant Physiology and Biochemistry*, 166 (2021): 328-340.
96. Wang, X. Q., Han, L. H., Zhou, W., Tao, M., Hu, Q. Q., Zhou, Y. N., Li, X. B., Li, D. D. & Huang, G. Q. "GhEIN3, a cotton (*Gossypium hirsutum*) homologue of AtEIN3, is involved in the regulation of plant salinity tolerance." *Plant Physiology and Biochemistry*, 143 (2019): 83-93.
97. Wang, Y., Zhang, G., Zhou, H., Yin, S., Li, Y., Ma, C., Chen, P., Sun, L. & Hao, F. "GhPYL9-5D and GhPYR1-A positively regulate *Arabidopsis* and cotton responses to ABA, drought, high salinity, and osmotic stress." *BMC Plant Biology*, 23 (2023): 310.
98. Wasilewska, A., Vlad, F., Sirichandra, C., Redko, Y., Jammes, F., Valon, C., Frey, N. F. D. & Leung, J. "An update on abscisic acid signaling in plants and more." *Molecular Plant*, 1 (2008): 198-217.
99. Wildermuth, M. C., Dewdney, J., Wu, G. & Ausubel, F. M. "Isochorismate synthase is required to synthesize salicylic acid for plant defence." *Nature*, 414 (2001): 562-565.
100. Xiong, X. P., Sun, S. C., Zhang, X. Y., Li, Y. J., Liu, F., Zhu, Q. H., Xue, F. & Sun, J. "GhWRKY70D13 regulates resistance to *Verticillium dahliae* in cotton through the ethylene and jasmonic acid signaling pathways." *Frontiers in Plant Science*, 11 (2020): 69.
101. Yan, J., Li, H., Li, Y., Zhang, N. & Zhang, S. "Abscisic acid synthesis and root water uptake contribute to exogenous methyl jasmonate-induced improved tomato drought resistance." *Plant Biotechnology Reports*, 16 (2022): 183-193.
102. Yan, M., Yu, X., Zhou, G., Sun, D., Hu, Y., Huang, C., Zheng, Q., Sun, N., Wu, J., Fu, Z, et al. "GhCDPK60 positively regulates drought stress tolerance in both transgenic *Arabidopsis* and cotton by regulating proline content and ROS level." *Frontiers in Plant Science*, 13 (2022): 1072584.
103. Yang, D. L., Li, Q., Deng, Y. W., Lou, Y. G., Wang, M. Y., Zhou, G. X., Zhang, Y. Y. & He, Z. H. "Altered disease development in the *eui* mutants and *Eui* overexpressors indicates that gibberellins negatively regulate rice basal disease resistance." *Molecular Plant*, 1 (2008): 528-537.
104. Zafar, M. M., Zhang, H., Ge, P., Iqbal, M. S., Muneeb, A., Parvaiz, A., Maqsood, J., Sarfraz, Z., Kassem, H. S., Ismail, H, et al. "Exploiting morphophysiological traits for yield improvement in upland cotton under salt stress." *Journal of Natural Fibers*, 20 (2023): 2282048.
105. Zahoor, R., Zhao, W., Dong, H., Snider, J. L., Abid, M. & Zhou, Z. "Potassium improves photosynthetic tolerance to and recovery from episodic drought stress in functional leaves of cotton (*Gossypium hirsutum* L.)." *Plant Physiology and Biochemistry*, 119 (2017): 21-32.
106. Zahoor, R., Zhao, W., Abid, M., Dong, H. & Zhou, Z. "Potassium application regulates nitrogen metabolism and osmotic adjustment in cotton (*Gossypium hirsutum* L.) functional leaf under drought stress." *Journal of Plant Physiology*, 215 (2017): 30-38.
107. Zhang, H., Shen, G., Kuppu, S., Gaxiola, R. & Payton, P. "Creating drought- and salt-tolerant cotton by overexpressing a vacuolar pyrophosphatase gene." *Plant Signaling & Behavior*, 6 (2011): 861-863.
108. Zhang, D., Li, J., Niu, X., Deng, C., Song, X., Li, W., Cheng, Z., Xu, Q., Zhang, B. & Guo, W. "GhANN1 modulates salinity tolerance by regulating ABA biosynthesis, ion homeostasis, and the phenylpropanoid pathway in cotton." *Environmental*

- and *Experimental Botany*, 185 (2021): 104427.
109. Zhang, D., Zhang, Z., Unver, T. & Zhang, B. "CRISPR/Cas: A powerful tool for gene function study and crop improvement." *Journal of Advanced Research*, 29 (2021): 207-221.
110. Zhang, H. J., Zhang, N., Yang, R. C., Wang, L., Sun, Q. Q., Li, D. B., Cao, Y. Y., Weeda, S., Zhao, B. & Ren, S. "Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA, and GA₄ interaction in cucumber (*Cucumis sativus* L)." *Journal of Pineal Research*, 57 (2014): 269-279.
111. Zhang, T., Hu, Y., Jiang, W., Fang, L., Guan, X., Chen, J., Zhang, J., Saski, C. A., Scheffler, B. E., Stelly, D. M, et al. "Sequencing of allotetraploid cotton (*Gossypium hirsutum* L. acc. TM-1) provides a resource for fiber improvement." *Nature Biotechnology*, 33 (2015): 531-537.
112. Zhang, X., Hu, D. P., Yuan, L., Yuan, C., Abidallha, E. H., Dong, Z. D., Chen, D. H. & Zhang, L. "Developmental and hormonal regulation of fiber quality in two natural-colored cotton cultivars." *Journal of Integrative Agriculture*, 16 (2017): 1720-1729.
113. Zhao, P., Lu, G. H. & Yang, Y. H. "Salicylic acid signaling and its role in responses to stresses in plants." *Mechanisms of Plant Hormone Signaling under Stress*, 39 (2017): 413-441.

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