

REVIEW

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Genetic and biotechnological innovations for drought and heat resilient cotton production: a review

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Abstract

Cotton (*Gossypium hirsutum* L.) is a primary fiber crop of the globe, especially in tropical and subtropical regions. Growth and development of crops are severely affected by climate change effects, especially drought and heat stress. Conventional breeding efforts have been practiced, incorporating heat and drought stress tolerance from tolerant crop genotypes or related tolerant crop species, which take many years until the development reaches the farmers' fields. Biotechnological innovations have made the process of genetic improvement fast, and even genes from unrelated crop species can be transferred to cotton in order to make it tolerant to deteriorating environmental stresses brought by climate change. In this review article, the consequences of heat and drought stress on cotton crops and their management options through biotechnological innovations like gene transformation, gene editing, epigenetic modifications, and their limitations are discussed. Overall, the objective of this review is to present a comprehensive analysis of biotechnological innovations aimed at heat and drought stress tolerance in cotton, along with future research thrusts.

Keywords Climate change, Abiotic stress, Cotton improvement, Genetic engineering

Background

Cotton (*Gossypium hirsutum* L.) is an important cash crop of the world and makes a major contribution to fulfilling fiber needs. Cotton is a crop adapted to tropical and subtropical climates, where China, India, the USA, Brazil, and Pakistan are the top five producers. The global value of the cotton market was around US\$43.00 billion in 2024 and is estimated to grow to \$55.57 billion in 2033 (Renub Research 2025). Global cotton production in 2024–2025 was 120 million bales, which is expected to decline to 117 million bales in 2025–2026 due to reduced acreage and

loss to abiotic stresses (Lanclos et al. 2025). The major reason for the decline in cotton yield is climate change, especially heat and drought stresses (Li et al. 2021; Zhou et al. 2024). Cotton needs plenty of water for its growth and development. Estimated water consumption (global average) to produce one kilogram of lint is 10 000 L of water (Hoekstra 2015), and to produce 24 million tons of lint, approximately 6.8 million m³ of fresh water is needed annually. Even by saving 25% of water, 1.7 million m³ of fresh water can be saved. This 25% water saving can be achieved by the combination of water-use-efficient cotton genotypes and efficient irrigation scheduling/reduced irrigation and has already been practically reported (Devi et al. 2020; Ahmad et al. 2021). Being a crop of tropical and subtropical regions, vegetative growth of cotton is moderately tolerant to drought and heat stresses, but reproductive development is highly sensitive to climatic variations (Ul-Allah et al. 2021; Zhang et al. 2023a). Masoomi-Aladizgeh et al. 2021) reported that 5-day

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exposure to 40 °C affects pollen fertility and reduces boll production up to 65%. Heat and drought stresses not only affect reproductive stages but also affect fiber quality (Gao et al. 2021; Ul-Allah et al. 2021). Climate change is taking place steadily, and the irreversible process, which is resulting in heat and drought stress in tropical and subtropical areas (Abdelrahim et al. 2026), may affect cotton production more badly in the future; therefore, it is time to address these urgent challenges.

Abiotic stresses such as heat and drought disrupt the biological processes in plant growth and development. Cotton plants are sensitive to drought stress, especially at the reproductive stage, where their water needs are maximum (Sun et al. 2021; Wu et al. 2024; Liu et al. 2025). Likewise, heat stress results in lower retention of boll and light boll weight, leading to reduced seed cotton yield (Iqbal et al. 2017; Bista et al. 2024; Liu et al. 2025). Lower boll retention coupled with smaller boll size under drought and heat stresses is attributed to complex molecular, physiological, and biochemical processes, leading to disruptions in carbohydrate and energy metabolism and thus less lint produced (Zhang et al. 2023b, 2024b). It is reported that the optimum day temperature required for growth and development of cotton is 28 °C with a variation of 2–3 °C, and cotton growth is hampered at day temperatures above 35 °C due to reduced Rubisco activity (Sharwood 2017; Smith et al. 2023). Not only daily maximum temperatures but also an increase in night temperatures have shown deleterious effects on cotton's metabolism. Loka et al. (2020) evaluated cotton's growth at 32/24 °C (day/night) and 32/30 °C temperatures and found that high night temperatures had a negative impact on photosynthetic rate, cell membrane integrity, and carbon metabolism. Therefore, any change in temperature, either day or night, might negatively influence the growth and development of cotton.

Researchers have developed various strategies to deal with heat and drought stresses, including exogenous use of osmoprotectants like proline, glycine betaine, aminobutyric acid, etc. (Ul-Allah et al. 2021; Nayak et al. 2023) and development of tolerant genotypes (Zafar et al. 2023; Prado et al. 2025). Exogenous use of osmoprotectants is not sustainable and puts extra economic pressure on the farmers, leading to a reduction in overall profitability. Use of tolerant genotypes is a sustainable solution to tackle the effects of climate change, but the development of new genotypes using conventional methods is a long and labor-intensive process. Moreover, tetraploid cotton has limited tolerance to heat and drought stresses (Khan et al. 2022), which alone cannot overcome the effects of climate change. These limitations have made it vital to investigate new sources of genetic variability regarding heat and

drought stress tolerance for climatic factors. Advancements in biotechnology and genetic engineering can overcome the crossing barriers for gene transfer among unrelated species (for example, the *Bt* gene from bacteria to cotton and the *phytoene synthase* gene (for the synthesis of vitamin A) from daffodils to rice have already been transferred using biotechnology) and open new avenues for the improvement of crop plants.

Genetic modifications of crop plants started in the 1980s with the successful transformation of tobacco and cotton by using *Agrobacterium tumefaciens* as the tool, and the first genetically modified cotton cultivar was introduced in 1996. This breakthrough opened new avenues in crop improvements, and gene transfer started across unrelated species. Initially, it was only transformation, but with more advances in biotechnology, researchers were able to alter gene expression by gene editing, especially by the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR-Cas9) technology. Both gene editing and genetic transformation have revolutionized cotton breeding via editing and introducing climate-resilient genes (Esmaeili et al. 2021; Ahmad et al. 2024). Moreover, targeted gene editing can be used to break unfavorable linkage and can create favorable linkage among the genes (Kumlehn et al. 2018; Sheri et al. 2025). Huang et al. (2022) reported that due to the allotetraploid nature of upland cotton, tight linkage among homoeologous genes makes it challenging to make recombination through conventional breeding. However, CRISPR/Cas-based genome editing enables the simultaneous modification of such linked loci, leading to improvement in complex traits without relying on repeated recombination.

Advancements in the collaborative research of breeding and biotechnology have made it possible to develop climate-resilient cotton genotypes in a shorter time than ever. Moreover, cotton biotechnology is applied in research where the benefits are directly delivered to farmers in terms of high profit by growing climate-resilient genotypes. In this review article, the effects of climate change, such as increased heat and drought stress scenarios, on cotton's growth and development, recent advances in cotton biotechnology for climate resilience, its limitations, and future research directions are discussed. This review will provide a framework for future research aimed at the development of climate-resilient cotton.

Effects of drought and heat stress on cotton growth

Drought stress and heat stress are environmental factors that affect the development of cotton plants. Effects of both stresses may be similar or, in some cases,

different. For example, under drought stress, the plant's transpiration needs are not fulfilled, and likewise, under heat stress, the plant increases transpiration to cool itself. In this section, the effect of heat and drought stresses on various attributes of cotton plants is discussed.

Morphological attributes

Plant morphology includes the shape, height, and size of plants and their parts like leaves, bolls, etc. Plant morphology plays a crucial role in the fixation of CO₂, as light interception is directly relevant to leaf areas exposed to light. Abiotic stresses such as heat and drought affect morphological attributes in plenty of ways. The first response to drought stress is triggered in roots. Cotton plants modify root system architecture in response to drought stress for efficient water extraction from the soil. Changes in the length of lateral roots, fewer new roots, and a lower angle of lateral roots in response to drought stress in drought-tolerant genotypes have been reported (Gao et al. 2023; Guo et al. 2024). A limited supply of water reduces the transpiration efficiency and photosynthesis, leading to reduced growth and development. The cotton plant is considered tolerant to moderate heat and drought stresses (Ul-Allah et al. 2021), especially at the vegetative stage, but at the reproductive stage, these stresses cause flower and boll shedding (Iqbal et al. 2017; Gao et al. 2021; Shani et al. 2025). Iqbal et al. (2017) reported up to 60% and 55% boll shedding due to heat and drought, respectively, and likewise, a 25% and 15% reduction in boll size. High night temperature increases the oxygen consumption by the plant to maintain the energy metabolism and limits carbohydrate delivery to buds and flowers, which leads to flower and bud shedding and production of a smaller number of seeds in the bolls (Reddy et al. 2020; Sharma et al. 2022). High temperatures not only affect the shoots but also the roots, where soil temperatures more than 33 °C can severely affect root growth by limiting the soil microbial activities and the function of temperature-sensitive enzymes (Xu et al. 2019; Arshad et al. 2021). Gao et al. (2021) investigated the effect of heat and drought stress on cotton yield and contributing traits. They found that both stresses resulted in yield loss, which was mainly due to boll shedding and reduction in boll size. Zafar et al. (2023) evaluated 12 cotton genotypes under heat, drought, and combined stress and reported significant reductions in plant height, the number of bolls per plant, boll weight, and seed cotton yield. They also reported that the combination of both stresses (i.e., heat and drought) caused greater damage to cotton plants compared with either stress alone. A severe reduction in plant morphological traits, i.e., plant height, the number of fruiting branches, the number of nodes per plant, and the ginning outturn of cotton due to heat stress

and drought stress, has been observed under field conditions (Anwar et al. 2022b). These losses can be minimized by devising strategies to mitigate the heat and drought effects. Liu et al. (2025) reported that heat and drought events in the last four decades contributed to a reduction in seed cotton yield and proposed urgent climate-resilient adaptation to cope with this problem. Consequences of heat and drought stresses on the cotton morphological attributes and seed cotton yield are summarized in Fig. 1.

Physiological and molecular attributes

Plant physiology refers to studies on internal physical and chemical processes, including photosynthesis, respiration, transpiration, ion homeostasis, and expression of genes involved in the regulation of biological processes for the growth and development of the plant. Drought and heat stresses limit the water uptake, which leads to hormonal imbalance and disturbances in plant metabolic processes.

Under drought and heat stresses, plants respond initially at the cellular level through cellular dehydration, osmotic imbalance, and protein destabilization (Saud et al. 2022). Heat and drought stresses lead to the rapid production of reactive oxygen species (ROS), which damage the cell and also act as signaling hormones (Zhou et al. 2016). In response to ROS production, a defense signaling mechanism in the cotton plant is activated, resulting in the production of abscisic acid (ABA). If the stress period elongates, ethylene production increases, leading to reduced growth and a reduction in fruiting points (Noreen et al. 2020; Qureshi et al. 2021). Nevertheless, early production of ROS serves as signaling hormones, but their increased production due to severe stress leads to oxidative damage of lipids, proteins, and nucleic acids, resulting in cellular and hormonal imbalance and dehydration. In response, plants increase transpiration to maintain optimal internal temperature and hydration, but when transpirational needs become greater than water uptake, it leads to plant wilting. All these processes reduce carboxylation efficiency and ultimately reduce production of assimilates (Bista et al. 2024; Liu et al. 2025). Wang et al. (2019a) reported that a short period of drought stress reduces the stomatal length and width by 3% and 4.5%, respectively, leading to reduced transpiration and photosynthesis. Bista et al. (2024) demonstrated that heat stress increases the leaf temperature by 9 °C, drought stress by 2 °C, and combined heat and drought stresses increase the leaf temperature by 11 °C compared with the control. They further reported that combined heat and drought stresses reduced the stomatal conductance, transpiration rate, and efficiency of Photosystem II by 97%, 84%, and 37%, respectively, compared with the control.

Increased heat and drought stresses affect plenty of physiological, metabolic, and molecular processes, which

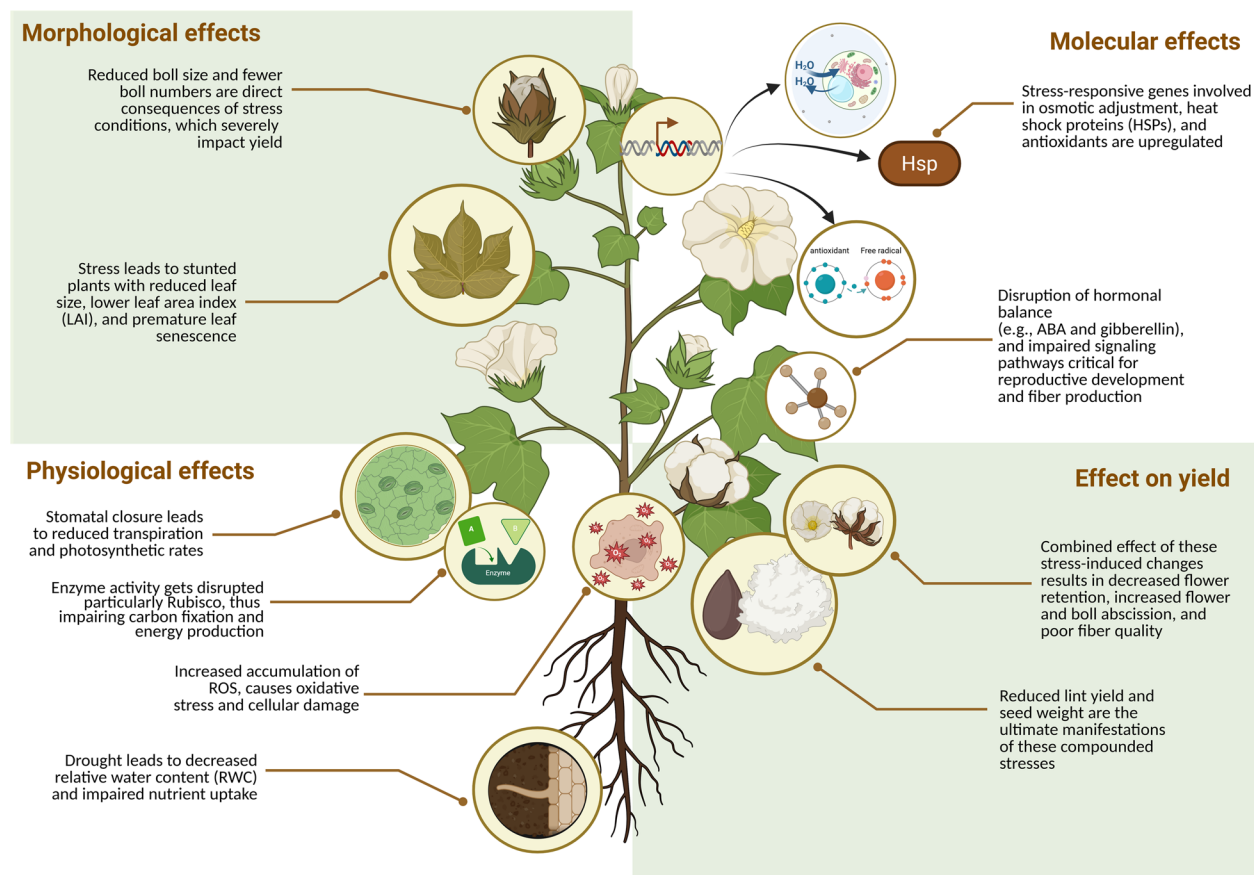


Fig. 1 Effects of heat and drought stresses on morphological, physiological, and molecular aspects of cotton plant leading to reduced yield and fiber quality

include chlorophyll and anthocyanin production, stomatal conductance, transpiration rate, electron transport, and antioxidant enzymatic activities. Reduced physiological efficiency, especially photosynthesis-related parameters, may be attributed to the activation of important enzymes like Rubisco, PEP carboxylase, ferredoxin-NADP⁺ reductase, etc., under heat and drought stresses (Carmo-Silva et al. 2012; Xu et al. 2022; Abro et al. 2023). The effects of heat and drought stresses on different physiological and molecular attributes are summarized in Fig. 1. In a crux, plant growth and development are dependent on the regulation of physiological and biochemical processes under stress conditions. Therefore, identification of genes controlling these processes and regulation of their expression are keys in developing climate resilient cotton genotypes.

Genetic improvement of abiotic stress tolerance in cotton

Genetic diversity is crucial for improving crops for different traits. Genetic diversity for heat and drought stress tolerance can be acquired from the genotypes of

the same species (intraspecific breeding) or related wild species (interspecific breeding). Improvement in cotton germplasm for heat and drought stress tolerance through the above-mentioned breeding techniques is discussed herewith.

Conventional techniques used for crop genetic improvement

Intraspecific breeding

Intraspecific breeding refers to breeding within the same species for the improvement of traits of interest using variations present in different genotypes. Various traits are associated with heat and drought stress tolerance, like osmotic adjustments, root attributes, plant development, and reproductive growth (flower and boll retention). Instead of an overall high temperature, cotton responds differently to high day and night temperatures. Warm nights lead to a reduction in cotton yield mainly due to high respiration requirements and fewer carbohydrates allocated to fiber development (Soliz et al. 2008; Khan et al. 2020; Engonopoulos et al. 2025). Therefore, in

developing the breeding strategies for heat and drought stress tolerance, all factors, i.e., short- and long-term drought, day and night temperatures, and the combination of both stresses, should be considered.

Climate-resilient and high-yielding genotypes with good fiber quality are the major objectives of any cotton breeding program. Conventional breeding programs are mainly based on hybridization of contrasting parental genotypes followed by selection. Various researchers have reported success in conventional breeding techniques (Singh et al. 2018; Anwar et al. 2022a; Bista et al. 2024). Minhas et al. (2018) reported a successful development of drought-tolerant cultivar “BH-67” at Cotton Research Station, Bahawalpur, Pakistan, by the cross of two *G. hirsutum* local varieties (VH-53 and BH-142) and its release for commercial use in drought-prone areas. Gören (2024) identified seven drought-tolerant genotypes from the generation of 28 crosses of *G. hirsutum* developed through a half-diallel crossing method at Adnan Menderes University, Faculty of Agriculture, Department of Field Crops, Aydın, Turkey. Despite reported success, challenges exist in intraspecific breeding, where the major challenge is the narrow genetic bases due to similar/same parentage of high-yielding and climate-resilient genotypes in the same region, which may lead to vulnerability of most genotypes to some epidemic situations. Therefore, there is a need to find ways to broaden the genetic bases of conventionally bred genotypes.

Interspecific breeding

Interspecific breeding refers to the breeding of two different species of cotton to improve the traits of interest. When desirable variation is not present in the native germplasm, breeders would find variation in the related wild and cultivated species, where crossing can be possible using various techniques. Heat and drought stress tolerance at reproductive growth has been reported in various *Gossypium* species like *G. arboreum*, *G. herbaceum*, *G. raimondii*, *G. tomentosum*, *G. thurberi*, etc. (Bibi et al. 2010; Stoilova et al. 2015; Iqbal et al. 2017). Stoilova et al. (2015) reported improved tolerance to heat and drought stress and improved fiber quality in interspecific hybrids relative to *G. hirsutum* cultivars. Iqbal et al. (2017) evaluated the advanced stable generations of interspecific hybrids and reported that they had higher flower and boll retention under heat and drought stresses compared with *G. hirsutum* cultivars. Magwanga et al. (2020) crossed *G. hirsutum* with *G. tomentosum*, and in segregating generations, they found five key genes associated with drought stress tolerance that were introgressed from *G. tomentosum*, which shows the potential of using wild relatives to improve abiotic stress

tolerance. Arslanova et al. (2025) reported the development of interspecific hybrids of *G. hirsutum* with two diploid species, i.e., *G. herbaceum* and *G. nelsonii*. Their findings highlighted a broadening of the genetic base of cotton germplasm, along with tolerance to biotic and abiotic stress, as well as improvement in the fiber quality. Likewise, Reddy et al. (2020) identified three interspecific lines (CS-T07, CS-B15sh, and CS-B18) derived from *G. hirsutum* and *G. tomentosum* with improved tolerance against heat and drought stress.

Despite the presence of genetic variability for climate resilience (especially heat and drought stress tolerance) in wild relatives of *G. hirsutum*, there are various challenges in introducing climate resilience traits from wild relatives to cultivated species, which include differences in ploidy level, instability of interspecific hybrids, reduced fertility, and difficulty in acquiring domesticated traits from *G. hirsutum* (Kaur et al. 2016; Anwar et al. 2022a). Conventional approaches are sustainable for improving the heat and drought stress tolerance in cotton, but at the same time, conventional methods face various challenges like lack of genetic diversity, a narrow genetic base, a long life cycle, etc., which make them time-consuming. Therefore, it is necessary to integrate conventional approaches with modern molecular approaches to improve the efficiency of the cotton breeding programs.

Biotechnological techniques

During the last two decades, the use of biotechnology in agriculture, especially in plant breeding, has increased, where it acts as a powerful tool to accelerate the process of crop improvement and the identification of desirable traits. There are various biotechnological tools being used for the improvement of cotton, including marker-assisted selection breeding (MAS), gene editing, epigenetic variations, and the development of transgenic cotton. All these tools have been discussed to address crossing barriers.

Molecular breeding

Molecular breeding refers to the use of molecular markers/genomic regions and gene expression. These markers are closely linked with the traits of interest. In modern breeding programs, high-throughput molecular markers such as single-nucleotide polymorphisms (SNPs) are predominantly used for selecting favorable traits and genotypes. SNP-based platforms, including genome-wide association studies (GWAS) and genomic selection (GA), have become central tools in cotton breeding pipelines. These approaches enable the identification of quantitative trait loci (QTLs) associated with yield and other complex traits and allow breeders to accelerate selection

through marker-assisted and genome-wide prediction strategies. Identification of QTLs facilitated the understanding of the genetics of quantitative traits, especially abiotic stress, and facilitated the selection process in breeding. Many traits are associated with drought and heat stress tolerance, including leaf morphology, leaf architecture, trichome density on leaves, canopy temperature, boll weight, and boll retention. Markers and QTLs linked with these traits under stress conditions are therefore considered important indicators of heat and drought tolerance.

Researchers have identified various QTLs linked with heat and drought stress tolerance in cotton. Jiang et al. (2000) mapped 40 QTLs related to leaf morphology using 260 restriction fragment length polymorphism (RFLP) markers and reported the highest number of leaf morphology (okra leaf type)-related QTLs on chromosome 15, where okra leaf type is associated with heat and drought stress. Magwanga et al. (2020) developed an interspecific backcross population (BC_2F_2) of 200 plants and, through linkage mapping, identified 30 stable QTLs associated with drought stress tolerance. Using genotyping-by-sequencing (GBS) with SNP and SSR markers, Shukla et al. (2021) constructed a genetic map in cotton and identified 19 QTLs for drought stress tolerance on nine different chromosomes. They found chlorophyll-related QTLs on chromosomes 3 and 8 and, likewise, relative water content-related QTLs on chromosomes 4, 8, and 12. Rani et al. (2022) identified 17 QTLs associated with different traits of heat stress tolerance in cotton, where individual QTLs account for 7%–35% variation in the specific traits. Likewise, Dwivedi et al. (2025) identified 46 expression sequence tag-SSR (EST-SSR) markers related to drought stress in cotton using transcriptomic data, out of which four markers were found only in highly drought-tolerant genotypes. They proposed that these four EST-SSR markers can be used for marker-assisted selection in cotton for drought stress tolerance. Most of the identified QTLs are associated with drought stress-related traits, while only a few studies have reported QTLs specifically linked to heat stress. However, since many stress-responsive traits overlap between heat and drought conditions, these QTLs may be considered relevant for both stresses.

Stress conditions alter the expression level of numerous different genes, and analyzing these changes in expression levels helps to identify genes that contribute to cotton adaptation under stress. Demirel et al. (2014) used 26 ESTs to study the heat-responsive genes and found four ESTs (*IAR3*, *FPGS3*, *GhHS126*, *GhHS218*) upregulated in response to heat stress, either short term or long term. In general, mitogen-activated protein kinase (MAPK) signaling pathways regulate the expression of

stress-responsive genes by activating or repressing specific transcription factors involved in stress-inducible transcription (Cowan et al. 2003; Zhang et al. 2018b; Zeng et al. 2025). Zhang et al. (2018b) found 38 potential MAPK modules involved in complicated crosstalk among metabolic processes to regulate the expression of stress-responsive genes. Systematic characterization of signal transduction pathways and involved genes can be crucial in developing climate-resilient cotton cultivars. The identified QTLs and genes can be used as a selection criterion in MAS to enhance the selection efficiency in developing heat- and drought-stress-tolerant cotton cultivars.

Gene editing and targeted mutations

After the identification of stress-related QTLs and genes, these genes can be used in molecular/marker-assisted selection for developing stress-tolerant genotypes. In recent years, gene editing to upregulate or downregulate the expression of stress-responsive genes has been increasingly employed (Wang et al. 2018; Sang et al. 2025), or sometimes these genes are silenced (Zhao et al. 2025). Gene editing is widely used to incorporate desired traits with precision into the cotton genome. Various gene editing technologies are being used, like clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR-Cas9), transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and homing endonucleases, which enable gene modifications by inducing double-strand breaks (DSBs) at specific DNA target sites (Khan et al. 2023). Plant cells' DNA repair machinery then fixes these breaks, which causes targeted gene alterations via two main pathways: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ frequently results in gene knockout or knockdown by generating indels, while HDR allows precise genome modification through the insertion or replacement of genes derived from either the same or a different organism (Gaj et al. 2016; Karavolias et al. 2021; Sheri et al. 2025).

Among these technologies, CRISPR-Cas9 is widely utilized in cotton due to its efficiency in inducing mutations in multiple target genes, a crucial requirement for the allotetraploid nature of the crop (Gao et al. 2017), while minimizing off-target effects (Zhu et al. 2018). In cotton, *Agrobacterium*-mediated transformation is primarily used to introduce the CRISPR system effectively (Chen et al. 2017; Long et al. 2018), while the virus-mediated transformation method is also practiced (Lei et al. 2022). So far, genome editing has been used in cotton to alter plant architecture (Sang et al. 2025), reduce seed toxicity (Lin et al. 2023; Li et al. 2024), improve disease resistance (Zhu et al. 2022), seed quality (Chen et al. 2021b), pest resistance (Fabrick et al. 2021; Ashraf et al. 2023),

and fiber yield (Yang et al. 2024b). However, gene editing studies on abiotic stress tolerance in cotton, especially drought and heat stress tolerance, remain very limited, while they have been explored more in other plants like *Arabidopsis* (Roca Paixão et al. 2019), rice (Usman et al. 2020), and tomato (Liu et al. 2020).

A recent study demonstrated the potential of a heat-inducible gene-editing system in cotton, where hypocotyl cuttings infiltrated with *Agrobacterium* carrying the CRISPR/Cas12b system were activated at three different high temperatures, resulting in successful mutations with larger deletions and base substitutions (Wang et al. 2020). This approach could facilitate the development of heat-resistant cotton by precisely targeting genes and pathways involved in the heat response while minimizing off-target effects. Another recently characterized stress-inducible GhRDL1 promoter in cotton is activated under polyethylene glycol-induced water deficit stress, with strong GUS expression observed in trichomes (Dass et al. 2017). This promoter could be valuable for overexpressing target genes under osmotic stress conditions and in trichomes. Recent

advancements in prime editing, an enhanced version of CRISPR technology, offer precise genome modifications without relying on the conventional double-strand break repair mechanism or donor templates. Despite its potential, prime editing has faced challenges in widespread application due to its lower efficiency. Moreover, redundant genes in the cotton tetraploid genome make transgene success challenging (Sheri et al. 2025). However, recent studies have shown improved efficiency and outcomes, surpassing earlier results of the gene-editing system, thereby offering renewed prospects for gene-editing in crops (Jiang et al. 2020; Norfaezah et al. 2024; Inam et al. 2024). The gene-editing process in crops is summarized in Fig. 2.

With progress in genome editing tools, desirable and precise genetic modification has become possible, and the regulation of native gene expression has become possible to improve crop performance under stress conditions. Researchers are working on DNA-free gene editing tools (Norfaezah et al. 2024), which may overcome the concerns associated with transgenic crops. There is a need to integrate different

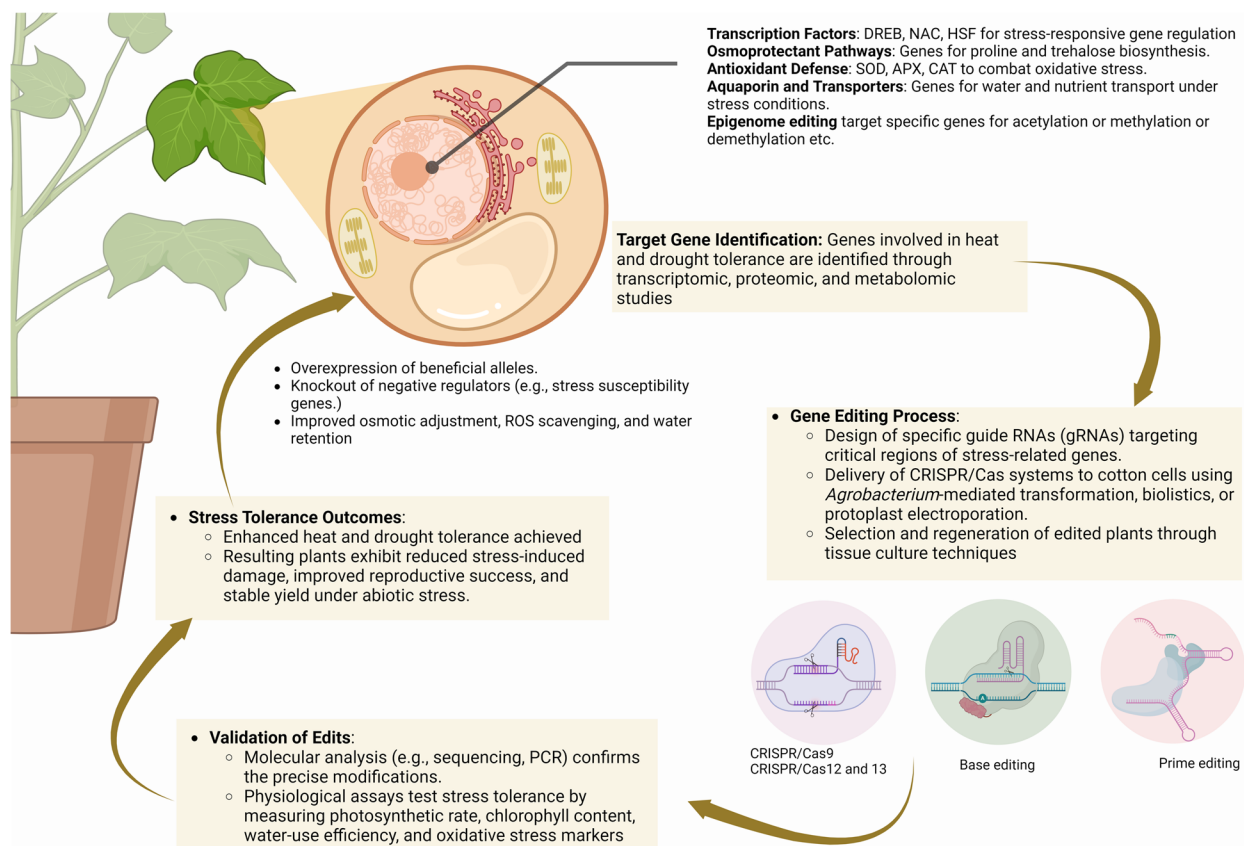


Fig. 2 Application of genome editing technologies to develop cotton plants with improved heat and drought stress tolerance through precise modifications of stress-related genes

conventional and biotechnological tools for sustainable cotton production in the changing climate.

Reverse genetics approach in cotton molecular breeding

Virus-induced gene silencing (VIGS), RNA interference (RNAi), and overexpression studies have provided valuable insights into the potential role of genes in drought and heat stress tolerance in cotton over the years (Mustafa et al. 2016; Tian et al. 2024a; Zeng et al. 2025). The VIGS of the *GOLDEN2-like* gene *GhGLK1* in cotton showed a significantly higher water loss rate and increased ion permeability in comparison to wild-type cotton under drought stress, indicating a potential of this gene in drought stress tolerance by affecting water-holding capacity and tolerance to reactive oxygen species (ROS), respectively (Liu et al. 2021). VIGS of another transcription factor (TF) gene, *GhTULP30*, resulted in more wilting compared with wild-type cotton under drought stress and a higher rate of cell death under trypan blue staining, showing the potential role of *GhTULP30* in increasing water-holding capacity and reducing cell damage (Li et al. 2022a). Hassan et al. (2021) showed that site-directed mutagenesis in universal stress protein (GUSP1) induced drought tolerance in transgenic cotton plants by increasing leaf relative water content (LRWC) and reducing solute leakage by increasing the cell membrane integrity. Another study revealed that silencing of the gene *GhMAP4K13* by VIGS results in drought and salt susceptibility in cotton (Zeng et al. 2025).

The reduced expression of *GhDRP1* that codes for type 2c-protein phosphatase by RNAi enhanced drought tolerance by improving the plant’s physiological attributes like relative water content, stomatal regulation, chlorophyll content, and oxidative stress tolerance (Chen et al. 2021c). Down-regulating the expression of *GhADF7*, a gene encoding an actin-depolymerizing factor (a member of the actin-binding proteins), was found to have a high seed germination rate and increased drought tolerance at the seedling stage (Qin et al. 2022). Similarly, several other key genes involved in drought stress response and heat stress tolerance from other transcriptional factors have been identified in cotton (Table 1), which can be optimized by using gene editing/VIGS to enhance the heat and drought stress tolerance in cotton.

Epigenetic approaches

Epigenetics refers to the heritable changes in gene expression due to different factors other than changes in DNA sequence, playing a crucial role in the development, adaptation, and domestication of crop plants. The phenotypic diversity and agronomic traits in cotton are significantly influenced by different epigenetic mechanisms like DNA methylation, histone modifications, and small RNA regulation. The adaptation ability of plants to environmental stresses such as heat and drought depends on epigenetic regulation. Notably, gene expression can be modified by these mechanisms without any DNA

Table 1 List of genes/transcription factors with potential functions in drought and heat stress tolerance in cotton

Gene/transcription factor	Role in drought and heat stress tolerance	Reference
<i>GhDnaJ94</i>	Improve stress tolerance by regulating the antioxidant defense mechanism	(Chen et al. 2025)
<i>GhGASA14</i>	Regulating the expression of oxidative stress-related genes through MAPK signaling	(Cai et al. 2025)
<i>GhCDPK16</i>	Induce thermotolerance by lowering ROS compound buildup and ABA signaling	(Lv et al. 2024)
<i>GhBLH5-A05</i>	Increased proline and chlorophyll content, and enhanced the expression of drought induced genes	(Zhang et al. 2024c)
<i>GhMYB44</i>	Overexpression of plants elevates the drought and heat stress tolerance by modulating stomatal aperture and improving the antioxidant enzymatic activities, thereby contributing to cellular protection under stress conditions	(Duan et al. 2023)
<i>GhMYB102</i>	Increased proline content and increased ABA biosynthesis	(Liu et al. 2023)
<i>GhVIP1</i>	Enhanced the expression of genes involved in proline biosynthesis	(Zhao et al. 2023)
<i>GhRF2-32</i>	It contributes to drought and heat stress tolerance by regulating oxidative stress homeostasis and enhancing the antioxidant enzymatic activities	(Gu et al. 2023)
<i>GhWRKY25</i> and <i>GhWRKY33</i>	<i>GhWRKY25</i> and <i>GhWRKY33</i> are involved in drought stress responses, where reduced expression improves the tolerance and post-stress recovery	(Ehsan et al. 2023)
<i>GhBEE3-Like</i>	Repression of <i>GhABF3-like</i> regulates stress-responsive signaling pathways, leading to increased expression of stress-related genes	(Chen et al. 2022)
<i>GhABF3</i>	Enhanced SOD and POD antioxidant enzyme activity, thus reducing oxidative damage	(Zhang et al. 2022)
<i>GhJUB1L1</i>	Regulate secondary cell wall synthesis and ion leakage	(Chen et al. 2021a)
<i>GhEXLB2</i>	<i>GhEXLB2</i> regulates osmotic stress tolerance by improving the root and hypocotyl growth under stress conditions	(Zhang et al. 2021)
<i>GhNAC072</i>	<i>GhNAC072</i> acts as a positive regulator of drought stress tolerance by maintaining water status, membrane integrity, and antioxidant defense	(Mehari et al. 2021)

sequence alteration, which allows plants to survive in stress conditions, having both immediate and long-term impacts on adaptation strategies.

Epigenetic modifications help to get ideas about cotton evolution and enhance the capability for crop improvement through epigenetic engineering. Histone modifications influence domestication traits by driving sub-genome divergence in a wild cotton species, *G. darwinii*. The A sub-genome exhibits higher active histone marks than the D sub-genome, which correlates with the expression biases (Han et al. 2023). Genome-wide studies have shown that dynamic DNA methylation reprogramming regulates fiber initiation and development, contributing to and regulating fiber development and fruiting branch formation, whose diversity contributes to genetic variation among cotton genotypes (Sun et al. 2018; Pei et al. 2025). Pei et al. (2025) also demonstrated that DNA N6-adenine methylation-mediated regulation of *GhMAF1* promotes fiber initiation, highlighting a direct epigenetic mechanism controlling fiber formation. Under abiotic stress conditions, epigenetic modifications regulate stress-responsive pathways, too. DNA methylation changes influence genes involved in ABA signaling and oxidative stress responses, thereby contributing to drought and heat tolerance (Yang et al. 2024a). In addition, histone modifications and small RNA-mediated pathways are known to modulate transcriptional responses to heat stress, enabling rapid activation of protective genes such as heat shock proteins and transcription factors (Shilpa et al. 2024). Together, these coordinated epigenetic mechanisms, including DNA methylation, histone modification, and small RNA regulation, fine-tune gene expression during fiber development and enhance cotton resilience to heat and drought stress.

Photoperiod sensitivity, which influences flowering and adaptation, is provided by epialleles like demethylated CONSTANS-LIKE 2 (COL2) (Song et al. 2017). DNA demethylase and methyltransferase genes regulate methylation under environmental stress, aiding stress response (Yang et al. 2018, 2019, 2024a). There are various types of epigenetic mechanisms to change the gene expression under stress, which play a key role in the adaptation of cotton to climate change. These mechanisms are elaborated in the following sections.

In response to abiotic stresses like heat and drought, DNA methylation plays an important role as an epigenetic modification by regulating gene expression. DNA methylation can notably change gene expression and chromosome interactions in plants for adapting to environmental stresses (Akhter et al. 2021; Rao et al. 2024). In the modification process, a methyl group is added to the DNA molecule, normally at cytosine bases, to

change the gene expression as a stress response (Singh et al. 2021; Wang et al. 2022; Liu et al. 2022). The circumstance and location of the methylation decide the suppression or activation of gene expression by DNA methylation (Akhter et al. 2021). To increase the ability of the cotton plants against an adverse environment, it has been shown that DNA methylation regulates gene expression in stress response (Singh et al. 2021; Yang et al. 2024a).

A histone deacetylase (GhHDT4D) suppresses the negative regulator GhWRKY33 via reduced H3K9 acetylation in cotton to improve drought tolerance (Zhang et al. 2020). Drought tolerance in Arabidopsis is amplified by the overexpression of *GhHDT4D*, while the expression of essential drought-response genes such as *GhDREB2A* and *GhRD29A* is reduced by silencing them in cotton. The studies by Zhang et al. (2020) underscore *GhHDT4D*'s significance in activating drought defense pathways. Lu et al. (2017) found changes in methylation in cotton using whole-genome bisulfite sequencing during drought stress and rewatering stages, which show hyper-methylation in mCpG, mCHG, and mCHH contexts and particularly sensitive methylation with CHH. Through microRNAs and hormone-related genes, long non-coding RNAs might influence drought response, which were found to control methylation patterns. These discoveries indicate the importance of epigenetic alterations in drought stress adaptation and improving crop production. Moreover, hormone-related genes have been connected to methylation changes, revealing the influence of DNA methylation in cotton stress tolerance (Lu et al. 2017; Kaya et al. 2024). Under heat stress, methylation alterations in cotton anthers indicate unique methylation patterns in CG, CHG, and CHH sites, with increased expression levels in promoter-unmethylated genes (Zhang et al. 2018a). It is also reported that demethylation of transposable elements and promoter methylation alterations by heat stress enhanced mitochondrial respiratory genes, which assist ATP synthesis for anther development. To influence the expression of drought-related genes, the demethylase GhALKBH10B controls the m⁶A levels. The m⁶A methylation patterns in mRNA can be modified by drought stress, with increased m⁶A levels corresponding to increased mRNA abundance. By regulating mRNA stability, this modification enhances drought tolerance, significantly in abscisic acid (ABA) and Ca²⁺ signaling pathway (Li et al. 2023). These findings emphasize the significance of epigenetic control in heat stress tolerance, which is important for enhancing cotton resilience.

“Chromatin remodeling” is another epigenetic approach, which refers to the modification of the chromatin structure to make it more or less accessible for transcription. Chromatin rearrangements can regulate gene activity by

opening or blocking access to DNA regions, which is triggered by stress conditions (Kong et al. 2020; Zhang et al. 2024a). Stress-mediated transcriptional modification is dependent on chromatin dynamics, including histone variant exchange and post-translational modifications. These molecular changes can either initiate the stress response or act further down the signaling pathway by occurring after stress-related transcription factors (Asensi-Fabado et al. 2017; Halder et al. 2022; Barragán-Rosillo et al. 2025).

MicroRNAs (miRNAs), small interfering RNAs (siRNAs), and long non-coding RNAs (lncRNAs) are all essential for the epigenetic modification in heat and drought stress response in cotton. Gene expression is controlled by these non-coding RNAs at both the transcription and post-transcriptional levels for plant adaptation to environmental challenges (Zhao et al. 2020; Jiang et al. 2025). miRNAs regulate gene expression in response to elevated temperature. Under heat stress, specific miRNA families, like miR2949, miR167, and miR160, modulate target gene expression to increase stress tolerance during different phases of cotton anther development (Chen et al. 2020; Ezin et al. 2023). The miRNAs modulate drought stress responses by targeting growth, development, and stress tolerance genes. Stress responses that improve plant drought resistance, including osmotic stress tolerance and antioxidant defense, are regulated by miRNAs. Table 2 shows how individual miRNAs in cotton are involved in heat and drought stress tolerance.

Apart from the mechanisms mentioned above, epigenetics is essential to develop stress memory and its transgenerational inheritance, which enhances plant resilience to future stresses (Forestan et al. 2020; Tian et al. 2024b; He et al. 2024). The phosphorylation-acetylation

interaction in modulating stress-related genes under heat and drought stress in cotton can be demonstrated using the epigenetic approach. Epigenetic variation is used to improve crop traits by epi-breeding, which makes it an efficient approach to create stress-tolerant cotton genotypes (Kakoulidou et al. 2021; Tonosaki et al. 2022).

The development of cotton by elevating stress resistance, production, and fiber quality without changing the genetic sequence can be possible through epigenetics. Researchers can use epigenome editing as well as other state-of-the-art methods to create cotton genotypes with more tolerance to abiotic stresses like drought and heat. This methodology can supplement standard breeding techniques to accelerate the generation of sustainable cotton cultivars. However, it is still hard to find stable epigenetic markers and to understand their generational inheritance process. Epigenetic approaches like DNA methylation, histone changes, and short RNAs show complexities that require cutting-edge techniques for successful manipulation. Nevertheless, epigenome-wide association studies (EWAS) have immense prospects to uncover advantageous epialleles and epigenetic markers for incorporation into breeding strategies. Further research and improved technologies will be fruitful in utilizing epigenetics fully in climate-resilient crop production.

Transgenic development

Transgenic crops became a reality more than 40 years ago with the pioneering idea of utilizing the T-DNA of *Agrobacterium tumefaciens*, a soil bacterium capable of inducing plant tumors (Grunewald et al. 2013). The first successful transformations of cotton were reported around the same time by Umbeck et al. (1987) and Firoozabady et al. (1987), expressing the kanamycin

Table 2 Different miRNAs identified in cotton that are associated with heat and drought stress

miRNA family	Stress type	Description	References
miR2949, miR167, miR160	Heat	Identified during the sporogenous cell proliferation stage; regulates high temperature stress response in cotton anthers	(Chen et al. 2020)
miR156, miR172	Heat	Identified during the meiotic phase; involved in high temperature stress response	(Chen et al. 2020)
miR393, miR3476	Heat	Identified during the pollen maturity stage; regulates high temperature stress response	(Chen et al. 2020)
miR398	Drought	Down-regulated in roots under drought stress	(Wang et al. 2013)
miR395	Drought	Down-regulated in leaves under drought stress	(Wang et al. 2013)
miR156-SPL2, miR162-DCL1, miR159-TCP3, miR395-APS1, miR396-GRF1	Drought	Show significant regulation relationships in roots and leaves under drought stress	(Wang et al. 2013)
miR750, miR2, miR14, miR276, miR279, miR-bantam, miR5176	Drought	Highly down-regulated in drought-tolerant cotton under water stress conditions	(Shriram et al. 2016)
miR160, miR408, miR393, miR319, miR394	Drought	Overexpression leads to drought tolerance in certain plants	(Zhou et al. 2013; Pan et al. 2018; Song et al. 2019; Hao et al. 2022; Jiang et al. 2022)

resistance gene. However, transgenic cotton (Bollgard) was only commercialized in 1996 with the introduction of the insect-resistant *Bt* gene in the USA, making it the third crop on the market after tobacco and tomato (James 1997). Since the introduction of *Bt* cotton, over 30 countries are growing transgenic cotton, achieving the highest global adoption rate among genetically modified (GM) crops, covering 76% of the total cotton-cultivated area, followed by soybean and maize (AgbioInvestor 2024). The widespread use of *Bt* cotton has effectively controlled serious outbreaks of cotton bollworm (*Helicoverpa armigera*) all over the world, and it drastically reduced the use of insecticides on the crops (Wu et al. 2008; Lu et al. 2022). Additionally, it has saved many farmers' lives from pesticide poisoning (Pray et al. 2002; Shahzad et al. 2024).

Currently, several transformation methods such as *Agrobacterium*-mediated transformation (Umbeck et al. 1987; Grunewald et al. 2013), microinjection (Gurusaravanan et al. 2020), particle bombardment (Rajasekaran 2013), and pollen tube pathway-mediated transformation (Wang et al. 2019b) are widely employed. These methods utilize various delivery approaches, including shoot apical-mediated transformation (Ge et al. 2023), *in planta* (Kalbande et al. 2016), and somatic embryogenesis (Srivastava et al. 2023). Among these, *Agrobacterium*-mediated transformation is the most widely used and reliable technique because it is simple, cost-effective, and efficiently transfers large DNA segments and low copies of transgenes into host plants (Srivastava et al. 2023). However, the success of *Agrobacterium* transformation is influenced by factors such as the strain of *Agrobacterium*, co-cultivation temperature, callus size, the duration of subculturing, market dynamics, and policy constraints (Sunilkumar et al. 2001; Khan et al. 2025). Since efficiency is highly dependent on genotype (Zhang 2023) and influenced by polyphenols produced by callus, as well as the conventional process being lengthy in obtaining transgenic lines, several advancements have been made over the years to address these challenges. Ge et al. (2023) developed a protocol using a shoot apical meristem cell-mediated transformation system, enabling the successful integration of transgenes into the genomes of recalcitrant cotton genotypes. Moreover, this approach reduces the transformation time from the conventional 12–24 months to approximately 8–9 months.

Despite significant advancements, none of the transgenic cotton varieties engineered for abiotic stress tolerance reached the market. This is likely due to several factors, such as stringent and time-intensive regulatory hurdles (Shukla et al. 2018), concerns regarding potential development of pest resistance and environmental safety

(Abbas 2018), and limited demand unless they promise to deliver remarkable benefits like drought tolerance or fiber quality and yield. Since the majority of the cotton-producing countries are facing the climate risk of having extreme temperatures like heat waves, prolonged drought periods, and even flooding, pyramiding of genes with potential functions in stress response and tolerance could be the most promising strategy in ensuring sustainable production and adapting to climate change (Sugumar et al. 2024). Several candidate genes have been explored in making transgenic cotton tolerant to drought and heat stress and combined drought and heat stresses so far, and most of them have been tested in *Arabidopsis* before introducing them into cotton, such as genes encoding vacuolar pumps (Gaxiola et al. 2001), transcriptional regulators, antioxidant enzymes, heat shock proteins, etc.

Transgenic cotton overexpressing *AVPI*, which encodes a vacuolar pyrophosphatase (H^+ -PPase) proton pump, has been shown to have increased drought and salt stress tolerance due to the upregulated activity of sequestering excess ions into the vacuole and thus lowering the solute potential. The plants also had well-developed root architecture with large lateral roots due to the enhanced synergistic effect of *AVPI* and auxin (Pasapula et al. 2011). Another study of cotton transformed with the *Thellungiella halophila* H^+ -PPase gene (*TsVP*) also showed consistent phenotypic results with *AVPI* (Zhang et al. 2016). A rice SUMO E3 ligase gene, *OsSIZ1*, which plays a vital role in regulating activities of enzymes that are involved in stress responses via post-translational mechanisms, when overexpressed alone in cotton, would drastically enhance drought and heat tolerance (Mishra et al. 2017). When both *AVPI* and *OsSIZ1* were co-overexpressed, the transgenic plants were found to produce significantly higher seed fiber yield and biomass compared with non-transgenic cotton or *AVPI*-overexpressing and *OsSIZ1*-overexpressing cotton plants under multiple stresses, such as combined drought and heat stress and combined drought and salt stresses, with an increase in photosynthetic rate and water use efficiency (Esmaeili et al. 2021). Similarly, when *AVPI* was co-overexpressed with *RCA*, a heat-stable variant that keeps Rubisco active as temperature increases by removing inhibitors through conformational changes, profoundly enhanced drought, salt, and heat tolerances of the transgenic plants (Smith et al. 2023).

Another way to engineer plants to cope with the adverse effects of abiotic stress involves targeting genes related to ROS homeostasis, as abiotic stress leads to excessive ROS production, which disrupts plant metabolism at cellular, biochemical, and physiological levels

(Mishra et al. 2023). Transgenic cotton plants overexpressing genes coding for superoxide dismutase (SOD) (Zhang et al. 2014), aldehyde dehydrogenase (ALDH) (Yang et al. 2016), and calcium-dependent protein kinase (CPDK) (Yan et al. 2022) were found to have enhanced growth under drought stress with an increase in proline and soluble sugars and reduced malondialdehyde (MDA) content, which help to reduce the damages to cellular structures such as membranes, proteins, and DNA, thus increasing plant yield under drought stress conditions.

Heat shock proteins are well-known molecular chaperones that not only help in folding or preventing misfolding of proteins in plants under stress conditions but also stabilize ROS homeostasis through up-regulation of the antioxidant system (Ul-Haq et al. 2019). Transgenic cotton overexpressing *HSP70* from *Agave sisalana*, a desert shrub, has been shown to increase heat-tolerance when the expression is induced by exogenous application of polyamine oxidase. The transgenic plants had significantly higher accumulation of proline and soluble sugars and increased chlorophyll content, resulting in larger boll size under heat stress conditions (Batcho et al. 2021). On the other hand, cold shock proteins (CSPs), which were previously thought to be involved solely in cold stress response as RNA chaperones that destabilize the secondary structures of RNA formed as the temperature drops and improve translational efficiency, were found to have much more significance in stress response (Sasaki et al. 2012; Keto-Timonen et al. 2016). Overexpression of a bacterial *DgCspC* gene in cotton resulted in significantly higher biomass and yield under drought and salt stress conditions compared with non-transgenic cotton, which was attributed to the upregulation of genes involved in proline and betaine synthesis, thus showing the potential of CSPs in osmotic and oxidative stress response (Xia et al. 2022). In addition to that, several transcription factor families, such as bZIP (Liang et al. 2016; Wang et al. 2017), WR1 (Liu et al. 2018), DREB (El-Esawi et al. 2019), and ZF-HD (Batool et al. 2023), were studied in generating transgenic cotton tolerant to drought stress. In another study by Mittal et al. (2014), transgenic cotton stacked with two transcriptional factor genes, *AtBIF5* and *AtRAV2*, made cotton more tolerant to drought stress with higher water-use efficiency and photosynthetic rates than its parents. The schematic diagram of the development of transgenic plants is presented in Fig. 3.

Over the years, advancements in transgenic cotton development have been driven by improvements in faster, genotype independent transformation methods, functional gene analysis through RNAi and VIGS techniques, as well as gene stacking to incorporate multiple traits. Recent efforts have shifted from targeting single abiotic stress factors to developing transgenic cotton varieties with enhanced tolerance to multiple stresses, ensuring greater resilience and

sustainability (Smith et al. 2023; Ahmad et al. 2024). However, stringent regulatory requirements and public concerns surrounding GM cotton continue to pose challenges. Addressing these concerns through rigorous safety assessments and transparent communication is essential for broader acceptance and commercialization.

Use of artificial intelligence (AI) in cotton breeding

Artificial intelligence (AI) assistance has recently emerged as a powerful tool to complement cotton breeding at the conventional and molecular levels, as it integrates machine learning for genomic prediction and high-throughput phenotyping. For example, AI-assisted genomic prediction models have been developed that analyze the whole-genome marker data for yield, stress tolerance, and fiber quality traits (Cheng et al. 2025) and thus offer new routes to accelerate selection in the cotton breeding programs. Li et al. (2022a, b) applied genomic prediction models to more than 1385 cotton breeding lines (F_4 to F_6 generation) from 42 experiments and achieved high cross-validation prediction accuracies for fiber quality and yield traits, demonstrating the potential of AI-assisted selection in breeding programs. Deep learning approaches, e.g., convolutional neural networks (CNNs), object detection models, recurrent neural networks (RNNs), etc., may be used for image-based phenotyping and real-time trait detection in cotton and can contribute to the automation of stress and disease monitoring (Ali et al. 2024; Yang et al. 2025). Rehman et al. (2025) used a deep learning model (The temporal logic of action and CNN) for cotton leaf insect pest and disease detection and achieved high accuracy (>90%) for aphids, armyworms, bacterial blight, and powdery mildew, thus highlighting the practical utility of AI in crop monitoring and management. Overall, the use of AI in decision-support systems with machine learning can shorten breeding cycles, leading to enhanced selection efficiency for complex heat- and drought-related traits.

Conclusion and future research thrust

Climate change is a reality, where heat waves and drought stress are major attributes of climate change that affect cotton productivity, and the intensity of heat waves and drought stress is always unpredictable. In most cases, heat wave and drought stress struck the cotton crop simultaneously, but most of the research deals with single stressor. The most sustainable way to deal with these stresses is to develop climate-resilient genotypes. Cotton breeding is a conventional way to develop crop genotypes, but narrow genetic variability limits the efficiency of conventional cotton breeding. Several genomic regions and genes associated with heat and drought stress tolerance have been identified that serve in molecular-assisted cotton breeding to improve resilience against stress. Advancements

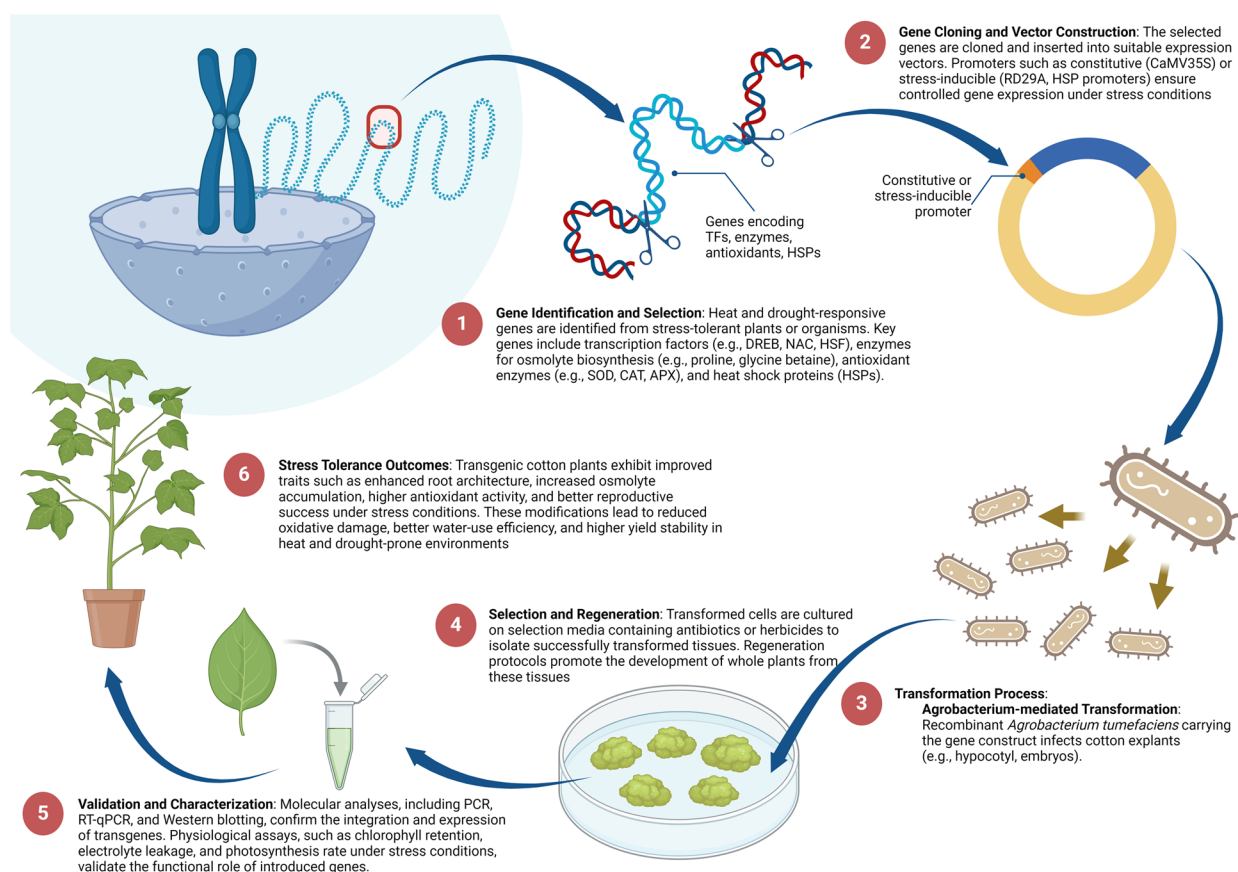


Fig. 3 Genetic transformation in cotton enhances heat and drought stress tolerance by incorporating stress-resilient genes through molecular interventions

in biotechnology have made it possible to transfer genes from unrelated species, which opens new ways to develop climate-resilient crops by introducing genes from heat- and drought-tolerant species. Moreover, gene editing and epigenetic approaches are also found helpful in improving the expression of stress-related genes for stress tolerance. Despite all this progress, to date, all the climate-resilient cotton genotypes have been developed by conventional breeding, and there is not a single GM climate-resilient cotton cultivar. Therefore, there is a dire need to develop interdisciplinary and integrative (conventional and molecular) strategies to develop climate-resilient cotton cultivars. Development of new techniques like shoot apical meristem transformation has made cotton transformation genotype independent, due to which high-yielding crop genotypes can be transformed for heat and drought tolerant genes and can be released back to the field directly, thus saving time. CRISPR/Cas-based genetic editing makes it possible to edit targeted genes with accuracy, whereby genes can be edited to induce tolerance against heat and drought stress. Advancements in DNA-free

genome editing using CRISPR/Cas9 ribonucleoprotein (RNP) complexes can rule out the ethical concerns related to GM cotton. Although biotechnological techniques (genome editing, epigenetic and transgenic) are precise and quick, there are many limitations associated with these techniques. These limitations include off-target modifications, non-uniform edits, formation of mosaics, and the effect on other associated genes. Along with technical limitations, ethical concerns also exist. All these modifications highlight the need for strong regulations for the development of transgenic crops.

Acknowledgements

We thank the International Institute of Education for sponsoring the first author as a visiting scholar through the Fulbright Program of the United States Education Foundation of Pakistan.

Authors' contributions

Conceptualization, funding acquisition, writing—original draft, review and editing: Ul-Allah S, Zhang H. Writing—original draft, review and editing, figure design: Sugumar T. Writing—original draft, review and editing, critical feedback: Jahin SA, Bhandari B. All authors have read and approved the final version of the manuscript.

Funding

This work was supported by the Texas State Support Committee and Cotton Incorporated (Grant No. 16-244TX), the CH Foundation (CH 21 No. T24G937-B53638-200), and the United States Department of Agriculture-National Institute of Food and Agriculture (Grant No. T24G937-B53638-200).

Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest. Author Zhang H is a member of the Editorial Board for Journal of Cotton Research. Author Zhang H was not involved in the journal's review of, or decision related to this manuscript.

Received: 1 September 2025 Accepted: 23 March 2026

Published online: 20 May 2026

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