

REVIEW

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Enhancing cotton resilience to challenging climates through genetic modifications

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Abstract

Cotton is one of the most important fiber crops that plays a vital role in the textile industry. Its production has been unstable over the years due to climate change induced biotic stresses such as insects, diseases, and weeds, as well as abiotic stresses including drought, salinity, heat, and cold. Traditional breeding methods have been used to breed climate resilient cotton, but it requires a considerable amount of time to enhance crop tolerance to insect pests and changing climatic conditions. A promising strategy for improving tolerance against these stresses is genetic engineering. This review article discusses the role of genetic engineering in cotton improvement. The essential concepts and techniques include genome editing via clustered regularly interspaced short palindromic repeats (CRISPR) / CRISPR-associated protein 9 (CRISPR-Cas9), overexpression of target genes, downregulation using RNA interference (RNAi), and virus-induced gene silencing (VIGS). Notably, the *Agrobacterium*-mediated transformation has made significant contributions to using these techniques for obtaining stable transgenic plants.

Keywords Cotton, Genome editing, Drought, Salinity, Heat

Background

Cotton is an important cash/fiber crop globally. It is the most widely used natural fiber in the world, and it is used to make a wide variety of products, including clothing, home furnishings, and industrial goods. Cotton is also a major source of income for many farmers and workers around the world. This versatility makes cotton a valuable commodity, and it is in high demand around the world (Liu et al., 2012a, b; Riello et al., 2011). It is

a relatively inexpensive fiber to produce, which makes it affordable for consumers. This affordability has helped to make cotton one of the most popular fibers in the world (Riello et al., 2011; Voora et al., 2020). It can be grown using sustainable practices, which helps to protect the environment. This sustainability is becoming increasingly important to consumers, and it is a major reason why cotton is a popular choice for clothing and other products (Liu et al., 2012a, b; Riello et al., 2011).

Over the past couple of decades, the impacts of industrialization, deforestation, and agricultural modernization have started appearing in the form of abrupt climatic changes that induce various biotic and abiotic stresses. Cotton has been facing multiple biotic and abiotic stresses. The major insects of cotton are bollworms (*Helicoverpa armigera* and *Helicoverpa zea*) (Wu, 2007), whiteflies (*Bemisia tabaci*) (Houndété et al., 2010), aphids (*Aphidoidea*) (Cao et al., 2008), spider mites (*Tetranychus* spp.) (Herron et al., 2004), cotton fleahoppers (*Pseudatomoscelis seriatus*) (Knutson et al., 2014), tarnished

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plant bugs (*Lygus lineolaris*), and pink bollworms (*Pectinophora gossypiella*) (Tabashnik et al., 2002). The insects could cause a 50% loss in production (Amjad Bashir et al., 2022). The major diseases such as Fusarium wilt (Cianchetta et al., 2015), Verticillium wilt (Cai et al., 2009), cotton leaf curl disease (Briddon et al., 2000), bacterial blight (Jalloul et al., 2015), alternaria leaf spot (Zhu et al., 2019), target spot (Sumabat et al., 2018), black root rot (Pereg, 2013), and stem canker (Laidou et al., 2000). The weeds also cause cotton yield reduction. The major weeds of cotton are Palmer amaranth (*Amaranthus palmeri*) (Berger et al., 2015), Johnsongrass (*Sorghum halepense*) (Wood et al., 2002), redroot pigweed (*Amaranthus retroflexus*) (Sharma et al., 2015), barnyard grass (*Echinochloa crus-galli*) (Bagavathiannan et al., 2013), goosegrass (*Eleusine indica*) (Wu et al., 2015), and yellow nutsedge (*Cyperus esculentus*) (Leon et al., 2003). The yield loss due to weeds can be from 40% to 85% (Nalini et al., 2015).

Major abiotic stresses and their impacts on cotton production

Significant global agricultural losses are caused by abiotic stressors including drought, salt, heat, and cold, which frequently impact crops with many stresses at once. These difficulties limit development, restrict plant growth, and lower yields, highlighting the necessity of understanding plant defense systems for increased resistance and food production when these effects intensify (Zhang et al., 2022).

Drought

The worldwide production of plants is significantly constrained by water unavailability that leads to drought stress. About 20% area of the world is facing drought stress (Rasheed et al., 2023), which cause 11% loss in overall production of crops (Riyazuddin et al., 2023), and 67% in cotton worldwide (Zafar et al., 2023). Drought-related yield losses exceed those brought on by additional external factors. Even though drought has an impact on plant development and metabolism, plants develop defenses to deal with it. For plants to adapt to the stress, signaling pathways and chemical reactions are involved in drought tolerance. At the cellular level, this involves the activation of several stress responses such as oxidative stress and stress signaling pathways. Abscisic acid (ABA) and transcription factors brought on by drought are essential in coordinating cotton's stress responses. Root formation, hormone production, photosynthesis, stomatal closure, and reactive oxygen species (ROS) scavenging are important drought stress responses in plants. A genetic resource for drought resistance is cotton, and sustained genetic advancements may be made using functional genomics and genome-altering methods like clustered regularly interspaced short palindromic repeats

(CRISPR) /CRISPR-associated protein 9 (CRISPR/Cas9) (Mahmood et al., 2020).

Salinity

Soil salinity is one of the major factors limiting crop yields around the globe, soil salinity affects could be 50% at $17.0 \text{ dS}\cdot\text{m}^{-1}$ salts in irrigated land and result in drastic reduction of crop yields (Negrão et al., 2016). It may be divided into two primary classifications: sodic soils, which include Na^+ containing salts, especially Na_2CO_3 , able to undergo alkaline hydrolysis, and saline soils, which are characterized by high amounts of soluble salts like NaCl and Na_2SO_4 and occasionally contain Cl^- and SO_4^{2-} of Ca^{2+} and Mg^{2+} . Most crops grow poorly in saline soils because there are too many neutral soluble salts, while sodic soils present difficulties because they are alkaline (Parihar et al., 2015). Although cotton has slight tolerance to salt, it is vulnerable to salt stress at important stages including germination, flowering, and boll development. Cotton plants under salt stress produce less biomass and fiber. Under saline conditions, the partial ion exclusion mechanism controls the absorption and accumulation of damaging ions (Na^+ and Cl^-) in cotton tissues. For cotton to be salt-tolerant, high K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ ratios must be maintained (Maryum et al., 2022).

Heat

Heat stress affects all the living organisms, especially plants which upsets cellular homeostasis and hinders growth, development, and survival. Due to their immobility, plants are always exposed to changing temperatures and other abiotic stresses. Heat stress, which commonly occurs together with drought and other stressors, is frequently held responsible for agricultural losses worldwide (Kotak et al., 2007). Plants use a variety of coping mechanisms, such as molecular adjustments and adaptive responses. Ion transporters, osmoprotectants, antioxidants, proteins, and signaling pathways all play their parts in these systems. When plants are under stress, they become aware of it, start to alter physically and biochemically, and turn on gene expression and metabolite production (Hasanuz-zaman et al., 2013). Temperatures that rise in the flowering season cause cotton to grow slower, lose flowers and have smaller bolls. This has major effect on yield, with a decrease of $110 \text{ kg}\cdot\text{hm}^{-2}$ of cotton production for every 1°C rise in temperature (Saleem et al., 2021).

Limitations of traditional breeding approaches

Conventional breeding has helped in improving the crop yields, but with the world population touching 7 billion and increasing with every coming day, the demands to increase food, feed, and shelter with the same pace is not possible

with old strategies. Traditional breeding methods can take many years to develop new varieties with desired traits. This is because the traits are transferred from one strain to another through sexual reproduction, which is a slow process (Moose et al., 2008). These methods can be imprecise, as they rely on the chance of recombination of genes. This can make it difficult to select specific traits, and it can also lead to the introduction of unwanted traits (Lyzenga et al., 2021). Sometimes the desired traits are not available among the crossable germplasms and it is not possible to cross incompatible species. This means that it can be difficult to introduce new traits into a crop or to improve existing traits that are not present in the gene pool (Sharma et al., 2013), and can be difficult to use to address complex traits, such as yield, disease resistance, and drought tolerance. This is because these traits are often controlled by multiple genes, and it can be difficult to select all of the genes simultaneously (Tanksley et al., 1997). It is very difficult to make interspecific crosses due to the low boll setting percentage, the number of seeds, germination of hybrid seeds, survival of plants, and fertility. If we overcome these difficulties, then backcrossing is also a major barrier which requires a lot of time and resources. Under these circumstances, genome editing and genetic engineering are viable tools for crop improvement when targeting the single trait such as drought, heat, salinity, and cold in elite lines. These methods can also be helpful when the genes for desired traits are not present in the gene pool.

Genetic engineering of crop plants

Recently a study showed that almost 17.2% of the world population lack healthy nutritional food due to the slow rate of conventional breeding which is 1.7% to produce fortified food (News, 2020; Ray et al., 2013). The time required for traditional breeding depends on the gene source's vicinity as well as the evolutionary relationship of the source species to the target crop. It can be 5 to 8 years for the primary gene pool and 10 to 15 years or more for the secondary or tertiary gene pool which is extremely time-consuming for a project to be accomplished and commercialized. Along with all these barriers sometimes successful hybridizing issues could be raised between source and recipient plant species which eliminates proper gene transferring (Jauhar, 2006).

An excellent method for asexually introducing well-characterized genes from unrelated organisms into plant cells is genetic engineering, which can regenerate complete plants with the inserted gene integrated into their genome. This approach can speed up the genetic improvement of crop plants and can sometimes take from less than a year to roughly 18 months. Additionally, this innovative method offers unrestricted access to the

gene pool without regard to the sexual compatibility (Altpeter et al., 1999).

Genetic modifications, whether through conventional breeding, genetic engineering, or genome editing, can improve an organism's ability to grow, reproduce, and yield better. Despite the fact that genetic engineering only transmits small segments of DNA, the resultant phenotype may result in the formation of an organism new to the current ecological network relationships (Wolfenbarger et al., 2000). Potential environmental consequences through invasiveness are dependent on existing opportunities for an introduced organism's unanticipated facility persistence, and gene flow; each of these, in turn, are dependent on many components of an organism's survival and reproduction (Pimentel et al., 2000). Following the techniques are being used in genome editing, CRISPR-Cas-based editing is an advanced technology with fast turnover time, sequence-targeted single-base specificity, and extreme sensitivity (Li et al., 2023). Post-transcriptional regulation of gene expression is mediated by RNA interference (RNAi), a widely conserved gene regulatory mechanism. Using dsRNA-based gene silencing, it is possible to modify crops genetically to bring about desired agronomic characteristics as well as disease resistance (Bharathi et al., 2023). Moreover, virus induced gene silencing (VIGS), an RNA-mediated reverse genetics technique and a vital method for investigating the role of genes, works by using posttranscriptional gene silencing mechanisms to downregulate endogenous genes and defend against systemic viral infections (Zulfiqar et al., 2023). *Agrobacterium*-mediated transformation mimics nature's process, facilitating successful genetic modification in crops, leading to improved traits across various crop species (Rahman et al., 2023). Several experiments were conducted to develop abiotic stress tolerant cotton (Table 1).

Developing abiotic stress tolerance in cotton using genetically-modified (GM) technology

Developing drought-tolerant cotton

The advanced method for plant functional genomics and molecular breeding research is CRISPR/Cas genome editing. Target genes have currently been modified by insertion, deletion, and base substitution through non-homologous end joining (NHEJ) using CRISPR/Cas9 genome editing technology (Sharif et al., 2019; Ullah et al., 2018). Transgenic plants with *AREB/ABF* overexpression demonstrated drought stress resistance through stomatal control as well as a better rate of photosynthesis. Transcription factors can be responsible for tolerance against drought, salt, and other abiotic stresses in cotton. For example, overexpressing *GhABF2* gene which encodes a bZIP transcription factor in cotton,

Table 1 List of genes responsible for the resistance against abiotic stresses (drought, salinity, heat, and cold)

No	Gene	Tolerance	Technique	Reference
1	<i>AREB/ABF</i>	Drought	CRISPR	(Ullah et al., 2018)
2	<i>Gh_A11G1801</i>	Drought	VIGS	(Gu et al., 2018)
3	<i>GhADF1</i>	Drought	RNAi	(Qin et al., 2022)
4	<i>DREB</i>	Drought	AMT	(Nasreen et al., 2021)
5	<i>GaZnF</i>	Drought	AMT	(Batool et al., 2023)
6	<i>LOSS</i>	Drought	AMT	(Yue et al., 2012)
7	<i>TaMnSOD</i>	Drought	AMT	(Zhang et al., 2014)
8	Isopentenyltransferase gene (<i>IPT</i>)	Drought	AMT	(Kuppu et al., 2013)
9	<i>PaKatA</i>	Drought	AMT	(Liu et al., 2019)
10	<i>AtRaV1</i> and <i>AtABI5</i>	Drought	AMT	(Fiene et al., 2017)
11	<i>GhABF2</i>	Drought	AMT	(Wang et al., 2021)
12	<i>HhERF2</i>	Drought	AMT	(Mittal et al., 2015)
13	<i>ApGSMT2g</i> and <i>ApDMT2g</i>	Drought	AMT	(Song et al., 2018)
14	<i>HSP70</i>	Drought	AMT	(Ni et al., 2021)
15	<i>GHSP26</i>	Drought	AMT	(Maqbool et al., 2009)
16	<i>DgCspC</i>	Drought	AMT	(Xia et al., 2022)
17	<i>ChCypI</i>	Salinity	Zinc finger technique	(Hou et al., 2022)
18	<i>HB12</i>	Salinity	CRISPR	(He et al., 2020)
19	<i>AVP1</i> , <i>gusA</i> , and <i>nptI</i>	Salinity	Silicon carbide whiskers	(Asad et al., 2008)
20	<i>IPT</i>	Salinity	AMT	(Liu et al., 2012a, b)
21	<i>betA</i> and <i>ah</i>	Salinity	AMT	(Lv et al., 2004)
22	<i>SbHKT1</i>	Salinity	AMT	(Guo et al., 2020)
23	<i>AtHDG11</i>	Salinity	AMT	(Yu et al., 2016)
24	<i>ApGSMT2g</i> and <i>ApDMT2g</i>	Salinity	AMT	(Song et al., 2018)
25	<i>AhCMO</i>	Salinity	AMT	(Zhang et al., 2009)
26	<i>AtNHX1</i> and <i>TsVP</i>	Salinity	AMT	(Cheng et al., 2018)
27	<i>AvDH1</i>	Salinity	AMT	(Chen et al., 2016)
28	<i>SNAC1</i>	Salinity	AMT	(Liu et al., 2014)
29	<i>AVP1</i>	Salinity	AMT	(Pasapula et al., 2011)
30	<i>AtSAP5</i>	Drought and heat stress	AMT	(Hozain et al., 2012)
31	<i>AsHSP70</i>	Heat	AMT	(Batcho et al., 2021)
32	<i>AmCBF1</i>	Cold stress	AMT	(Lu et al., 2022)
33	<i>AmDUF1517</i>	Cold stress	AMT	(Hao et al., 2018)
34	<i>GhDREB1B</i>	Chilling stress	AMT	(Wang et al., 2021)
35	<i>GhKCS13</i>	Cold stress	AMT	(Wang et al., 2020a, b)

AMT *Agrobacterium*-mediated transformation, CRISPR clustered regularly interspaced short palindromic repeats, RNAi RNA interference, VIGS virus-induced gene silencing

the genetically modified cotton expressed drought and salt tolerance via the regulation of ABA-related genes in cotton. Overexpression of *GhABF2* gene in cotton also showed higher yield compared with cotton which was not transformed (Ullah et al., 2018). Due to their significant function in the control of drought stress, WRKY transcription factors in cotton have received a lot of attention (Gu et al., 2018). Thirty-four IId WRKY genes were discovered in the *Gossypium hirsutum* genome, ten of these genes showed differential expression in response

to salt and drought stressors. VIGS technology was used to silence the highly expressed gene, *Gh_A11G1801*, in cotton plants. Cotton seedlings exhibited increased sensitivity to drought stress as a result of silencing. Additionally, these plants had more malondialdehyde (MDA) and less catalase (CAT) activity (Gu et al., 2018). Similarly, under abiotic stress, cotton's group III WRKY gene expression was evaluated. Under the effects of ABA, mannitol, and salt treatments, the expression levels of *GhWRKY7*, *GhWRKY50*, *GhWRKY59*, *GhWRKY60*, and

GhWRKY102 were dramatically elevated. It demonstrates that these genes may control pathways that respond to salt and/or drought stress, such as the ABA signaling system in cotton plants. Under conditions of high concentrations of mannitol and NaCl (200 mmol·L⁻¹), the genes *GhWRKY7* and *GhWRKY7102* were highly expressed in roots, demonstrating their critical function in root development (Yan et al., 2015). The GhWRKY1-like transcription factor was discovered in *G. hirsutum* as a regulator of drought resistance. By modifying ABA production and its interactions with several *cis*-elements, *GhWRKY1-like* overexpression in *Arabidopsis* increased drought tolerance (Hu et al., 2021).

Another study found that transgenic cotton with *AtABI5* overexpression was more resistant to drought stress in the field and greenhouse. Transgenic plants demonstrated better photosynthesis and water utilization efficiency. Transgenic plants also showed improved photo-assimilation and root and shoot sink capacities (Mittal et al., 2014). The protein encoded by *Gh_D01G0514* (*GhNAC072*) was in the nucleus and cytoplasm, a yeast two hybrid (Y2H) assay revealed that this protein has the potential of auto-activation, which was highly upregulated in both tissues. Its validation was carried out using VIGS experiment, the results revealed that VIGS plants have higher excised water loss and ion leakage than control plants (Liang et al., 2016a). bZIP transcription factor expression in an ectopic manner produced resistance in cotton to drought, and it was enhanced by *ABF2D*. *ABF2D* increases the photosynthetic productivity and stomatal control to increase drought tolerance (Kerr et al., 2018). When downregulated by RNAi, the actin depolymerizing factor *GhADF1* improves cotton's ability to withstand drought, leading to better root development, antioxidant enzyme activity, water-use effectiveness, and fiber production, making it a desirable option for crop improvement (Qin et al., 2022).

DREB gene under the control of the CaMV 35S promoter was expressed in cotton. The transgenic plants were tolerant to drought stress (Nasreen et al., 2021). When *GaZnF* gene was effectively introduced into cotton by *Agrobacterium*-mediated transformation, its expression level was increased under drought stress. Improved drought-resistance capabilities in transgenic cotton plants pointed to the possibility of producing drought-resistant cotton (Batool et al., 2023). Cotton's potential to withstand drought was improved by introducing the *Arabidopsis* gene *LOSS*, this decreased water loss while raising ABA level, gene expression, and membrane integrity. By increasing ABA production and physiological adaptations, *LOSS* overexpression improves cotton's resistance to drought (Yue et al., 2012). *TaMnSOD* gene from *Tamarix albiflorum* was used to improve cotton's

tolerance to drought, which resulted in better physiological and biochemical characteristics, greater antioxidant enzyme activity, better cell membrane integrity, and improved growth after recovering from stress. *TaMnSOD* overexpression promoted the growth of the root and leaf systems while improving superoxide scavenging, hence strengthening cotton's resistance to drought stress (Zhang et al., 2014). *IPT* gene is introduced through the PSARK promoter, which is water-deficit sensitive and maturation-specific, to increase cotton's resistance to drought. PSARK::*IPT* GM cotton exhibited delayed senescence, better biomass, decreased flower loss, preserved chlorophyll content, and higher photosynthesis under the shortages of water, demonstrating its potential to considerably increase drought resistance (Kuppu et al., 2013).

By expressing the CAT gene *PaKatA* from *Pseudomonas aeruginosa*, cotton yield, and drought resistance were increased. Transgenic plants produced a 34% greater field production under drought-like circumstances and showed increased activity of enzymes, proline content, water content, and decreased oxidative stress indicators, suggesting the possibility for cotton and other crops to become more productive and drought-resistant (Liu et al., 2019). Genes from *Arabidopsis* were expressed in transgenic cotton. Improved photosynthesis, greater drought tolerance, and water efficiency were all exhibited by *AtRAV1/2* and *AtABI5* transgenic plants. These plants showed improved drought resistance in greenhouse and field conditions, deeper roots, bigger leaves, and greater scavenging of ROS, suggesting the potential for cotton and other crops (Mittal et al., 2014). With higher water usage efficiency (WUE), transgenic cotton lines overexpressing *AtRAV1* or *AtABI5* showed better ABA responses. Under drought circumstances, these plants showed decreased ABA levels, increased stomatal conductance, faster photosynthesis, and better intrinsic WUE, indicating potential advantages for cotton production through greater drought resilience (Fiene et al., 2017). Cotton transcription factor *GhABF2* is essential for abiotic stress signaling; its overexpression increases tolerance to osmotic and salt stress, while its silence increases vulnerability. A sustainable method for improving cotton's resilience to salt and drought stress is through biotechnology manipulation of *GhABF2*. *PeDREB2a* and *HhERF2* were introduced into cotton to increase drought and high-salt tolerance, seed germination, yield, and agronomic characteristics (Mittal et al., 2015).

A cytoplasmic *HSP70* gene called *GhHSP70-26* improves cotton and tobacco plants' ability to withstand drought. Overexpression decreases wilting, while enhancing survival, root development, and chlorophyll content. Transgenic plants showed lower amounts of MDA and ROS, and increased levels of proline content, superoxide

dismutase (SOD) activity, and peroxidase (POD) activity. Silencing causes membrane damage, wilted leaves, and increased water loss. By minimizing cell and membrane damage, GhHSP70-26 interacts with GhAPX2 and is controlled by GhbZIP43 and GhHSF8. This protein is essential for the response to drought (Ni et al., 2021). The *GHSP26* gene from *G. arboreum*, a small heat-shock protein gene, was introduced into *G. hirsutum* plants to improve drought tolerance. Transgenic plants exhibited enhanced drought tolerance, indicating the involvement of *GHSP26* in plant response to drought stress (Maqbool et al., 2009). Cotton has improved stress resistance due to the gene *DgCspC*, which codes for a bacterial cold shock protein. In addition to promoting cotton growth and enhancing its ability to withstand salt and drought, heterologous expression of *DgCspC* additionally enhances photosynthetic efficiency and yield. *DgCspC* improves the accumulation of chemicals linked to stress, osmotic control, and ROS scavenging (Xia et al., 2022).

Salt tolerance

To cope with salinity stress, genome editing technologies provide easy way which can prove a powerful tool to avoid yield loss. Many experiments have been successful done to develop salt resistant cotton (Table 1). Through the use of VIGS technology, *GhWRKY6-like* gene was silenced in cotton, making cotton plants more susceptible to salt and drought stress (Ullah et al., 2018). *ChCypI* gene was annotated through the zinc finger technique in cotton which resulted in the regulation of the expression of salt-tolerance related genes in cotton (Hou et al., 2022). Cotton was genetically modified through *GhABF2* gene to improve drought and salt tolerance, enhance fiber production, and higher proline content (Liang et al., 2016b). CRISPR/Cas genome editing of *HB12* gene boosted cotton resistance to drought by inhibiting the expression of ABA-responsive genes and stress-related genes (*ABI2*, *DREB2A*, *RD29A*, *RD22*, *RD26*, *RD28*, *SOS1*, *SOS2*, *NHX1*, and *HKT*). *GhHB12* overexpression in cotton and *Arabidopsis* reduced ABA sensitivity and decreased salt/drought tolerance. Cotton has more tolerance for abiotic stress when *GhHB12* is down-regulated. *GhHB12* supported abiotic stress tolerance and plant development by collaborating with other transcription factors, kinases, and enzymes (He et al., 2020).

For cotton, a silicon carbide whisker-mediated gene transfer method with the ability to recover viable and reliable transformants was created. *AVPI*, *gusA*, and *nptI* gene was introduced in cotton through silicon carbide whiskers, and genetically transformed cotton showed tolerance against salinity (Asad et al., 2008). The introduction of the *IPT* gene in cotton through the latest genetic engineering method showed high salt tolerance (Luo et al., 2001).

Cotton plants that expressed the *IPT* gene via the *Ghcyp* promoter had slower leaf senescence and better resilience to environmental challenges. The transgenic lines showed higher levels of cytokinin and chlorophyll, as well as better ROS-scavenging enzyme activity and growth traits. Comparing transgenic lines with non-transformed lines under salt stress, the transgenic lines showed increased biomass and endogenous cytokinin levels. The transgenic lines also had better fiber quality and lint production (Liu et al., 2012a, b). The modified plants showed increased tolerance to salt and herbicide resistance. The *betA* and *ah* genes were inserted into superior cotton cultivars, resulting in transgenic plants and progeny with improved characteristics (Yin et al., 2004). Under salt stress, transgenic cotton co-expressing *ApGSM2g* and *ApDMT2g* genes showed higher salt tolerance, osmoregulation, and K^+/Na^+ balance, which improved photosystem II (PSII) performance, photosynthesis, growth, and seed output. The salt tolerance and production of cotton in saline areas may be improved with this approach (Song et al., 2018). With improved potassium absorption, K^+/Na^+ balance, and antioxidant activity, cotton salt tolerance is improved by *SbHKT1* gene overexpression. Better potassium absorption and effective regulation of ROS improve germination, biomass, and root development, and reduce sodium levels when exposed to salt stress (Guo et al., 2020).

It has been demonstrated that the *Arabidopsis* transcription factor *AtHDG11* improves salt and drought tolerance in a variety of plant species, including cotton and poplar. *AtHDG11*-expressing transgenic cotton and poplar plants showed greater tolerance to salt and drought stress as well as improved root growth (Yu et al., 2016). By raising glycine betaine (GB) levels, protecting cell membranes, and increasing photosynthesis, *AhCMO* gene from *Atriplex hortensis* increased salt tolerance in transgenic cotton. The potential of *AhCMO* overexpression for improving cotton growth under saline environments is indicated by field data that revealed better yields in salty conditions (Zhang et al., 2009). Cotton that co-expresses *AtNHX1* and *TsVP* exhibits improved salt tolerance, germination, growth, and seed yield under salt stress. Because of their better ion balance and osmotic potential, transgenic plants survive under salty environments, significantly increasing cotton's salt resistance (Cheng et al., 2018). Overexpression of *AvDHI* gene improves salt tolerance by decreasing membrane ion leakage and raising SOD activity. *AvDHI*-expressing transgenic cotton lines were better than wild-type plants in terms of boll count, boll weight, and seed cotton yield in soils with high salt content (Chen et al., 2016). Salt tolerance is improved in transgenic cotton with more GB

accumulation. Transgenic lines with higher GB levels have better germination, seedling development, and overall plant performance when exposed to salt stress. In saline soil, transgenic lines exhibit higher seed cotton yield compared with wild-type plants (Zhang et al., 2011a, b). Cotton with *SNAC1* gene overexpressed grows roots rapidly, transpires less and is more resilient to salt and drought stresses. Cotton plants that overexpress *SNAC1* develop rapidly have higher proline content, less MDA, and more bolls, demonstrating their greater stress resistance (Liu et al., 2014). By enhancing the proton electrochemical gradient, making it easier to store ions and sugars in vacuoles, and encouraging auxin transport for larger root systems, overexpression of *AVPI* gene improves salt and drought tolerance in a variety of plant species. Cotton plants that express *AVPI* grow vigorously in high-salt and low-water environments, increased drought and salt stress tolerance in greenhouse conditions, and produce more fiber in dry-land field conditions (Pasapula et al., 2011).

Heat and cold tolerance

The heat shock protein gene (*AsHSP70*) was used to transform *G. hirsutum*. Under heat stress, transgenic cotton plants showed enhanced *AsHSP70* expression, particularly in the leaves. In comparison to control plants, they exhibited increased membrane stability, decreased electrolyte leakage, and less membrane damage. Significant differences in the contents of proline, soluble sugars, and chlorophyll were also seen in transgenic plants. Transgenic plants in the field showed improved boll development. These results showed the potential of *AsHSP70* for increased heat and other abiotic stress tolerance in cotton (Batcho et al., 2021). A recently developed genome editing technology with a modest size and few off-target effects is called CRISPR/Cas12b (*C2c1*). It is ideal for editing high-temperature-resistant plant species like cotton since it is temperature-inducible. Through *Agrobacterium*-mediated transformation, upland cotton mutants were effectively created using CRISPR/Cas12b, with the best results being obtained at 45 °C for 4 days (Wang et al., 2020a, b). By activating stress-responsive genes, ectopic expression of *AtSAP5* gene in transgenic cotton improves tolerance to drought and heat stresses. Compared with wild-type plants, transgenic plants had better photosynthesis, seedling development, and leaf viability. The improved drought tolerance is a result of the preservation of PSII complexes and metabolic activities.

According to the study, enhancing the production of cotton in semi-arid areas with severe drought and heat stress may be possible by expressing *SAP* genes (Hozain et al., 2012). *AmCBF1* from *Ammopiptanthus mongolicus* was introduced into upland cotton to increase tolerance

to drought and cold stress. Higher relative water content, more chlorophyll and soluble sugars, and less ion leakage were all characteristics of transgenic plants. Transgenic lines had different morphological traits from the control, such as decreased epidermal cell area, stomatal density, and root-to-shoot ratio. Transgenic plants had increased net photosynthetic rate, stomatal conductance, and transpiration rate despite decreased photosynthetic capacity (Lu et al., 2022). Transgenic plants with enhanced tolerance to salt, drought, and cold stresses were produced as a result of the introduction of *AmDUF1517* into cotton. Higher antioxidant enzyme activity and decreased ROS accumulation in Trans-*AmDUF1517* cotton point to better ROS homeostasis and reduced cell membrane damage. These results suggest *AmDUF1517* as a candidate gene that may improve cotton's ability to withstand abiotic stress (Hao et al., 2018).

GhDREB1B increases the ability of cotton seedlings to withstand cold temperatures. Transgenic cotton becomes susceptible to chilling stress when *GhDREB1B* is silenced. Major cold-responsive genes, including those involved in fatty acids, lipid proteins, osmoprotection, and antioxidant enzymes, are activated by GhDREB1B. In contrast to wild-type, transgenic plant shows less MDA buildup and ion leakage, indicating improved chilling tolerance. Additionally, it builds up more free proline and soluble carbohydrates. *GhDREB1B* functions as a positive regulator, changing the expression of genes associated with low-temperature stress and improving cotton's resistance to chilling (Wang et al., 2021). *GhKCS13*, discovered in cotton by RNA-seq research, participates in the response to cold stress. Transgenic plants are more susceptible to cold stress when *GhKCS13* is overexpressed. The cold stress response is mediated by lipid remodeling, which includes sphingolipids and glycerolipids, as well as lipid-derived jasmonic acid (JA). Cotton's reaction to cold stress is controlled by complex interactions between lipid elements and JA (Wang et al., 2020a, b).

Implications for sustainable cotton production

In dry regions where crops are mainly dependent on rainwater that quickly evaporates and creates drought conditions, the farmers have to manage artificial irrigation that reduces profits. GM approaches, for instance, incorporating different genes increases production under harsh conditions such as introduction of *IPT* gene into cotton increased 30%–35% yield under harsh conditions (Kuppu et al., 2013), *betA* gene increased yield up to 3%–12% (Lv et al., 2007), and *AVPI* up to 20% (Zhang et al., 2011b, a). Transgenic plants not only increase yield but also affect fiber quality as well as other chemicals like proline content and sugar levels, etc. (Zhang et al.,

2011a, b). Similarly, salinity and other abiotic stresses are major causes of yield reduction especially in cotton. For salt stress, GM cotton with *AvDHI* can boost production by more than 32.5% (Yang et al., 2016). These advanced technologies provide a cheap way to cope with abiotic stresses with minimum loss of yield while elevating the overall profit at the farmer level.

Future prospective

Widespread restrictions on GM crops have been implemented in a number of countries because of concerns about human health, insect resistance, insufficient safety research, and poor scientific communication by seed producers. Moreover, GM crops can also produce antibiotic-resistant bacterial strains, enhanced amounts of heavy metals, and may be less nutritious. Another big issue about GM crops is misinformation in media which cause reduction in the acceptance of these crops. The extensive use of GM cotton may lead to a decrease in the diversity of cotton varieties, requiring crop programs with broad genetic bases to be sustainable in the face of changing environment. There is no precise data about affects of GM crops and this debate will never end. Most countries are now accepting the GM crops.

In recent years, climate has drastically changed which significantly altered the weather pattern and uneven rainfalls that result drought stress in crops (Meshram et al., 2022). All abiotic stresses may be happen in some areas, but rising temperature is a global issue that affects plants all over the world. According to Khan et al. (2013), an average 0.3 °C increase per decade and the temperature could be enhanced up to 3 °C till 2100. The yield reduced by 17% on every 1 °C increase in temperature. So, it is most important to work on heat stress, but still, there is not enough research on the withstand of cotton to higher temperatures.

For crops to successfully withstand several abiotic challenges, combining many genes is necessary, requiring a comprehensive approach that goes beyond single-gene modification (Esmaili et al., 2021). To cope with multiple stresses, co-overexpression of different genes is required to make plant resistant to multiple stresses, i.e., by co-overexpression of *RCA* and *AVPI*, the cotton is resistant to salt, drought, heat stress, and fibre quality is also improved (Smith et al., 2023). Through improving resistance to various stresses by targeted gene selection and pyramiding, the yield will be improved in the future (Esmaili et al., 2023).

Things are happening that are exciting in the realm of cotton's future! Scientists are developing new methods to improve cotton. They are altering its DNA to aid in its defense against pests and diseases, so that growers won't need to apply as many insecticides. Additionally, they

employ microscopic particles to strengthen and color cotton fibers. Farmers can care for their cotton crops by utilizing the proper quantity of water and fertilizers because of drones and satellites. There is yet more to discover! Scientists are attempting to add more nutrients and other beneficial ingredients to cottonseeds. Additionally, they are aiming to strengthen cotton plants to ensure they can withstand extremely cold or hot temperatures. And they're attempting to show how cotton plants utilize nutrients and water properly. To create stronger, longer fibers for clothing, they seek to improve cotton itself. Even though it's all really interesting, scientists are also taking care to consider how these changes can damage the environment. They are considering the safety of both people and animals as well as the environment.

Conclusion

Genome editing is a potential method for enhancing cotton's resistance to challenging environmental factors. Researchers have discovered techniques to make cotton more resilient to problems like drought and excessive salt concentrations by modifying the plant's genetic material. With the help of genetic engineering, the resistance against different abiotic stresses such as drought, salinity, heat, and cold can be achieved with less time and more efficiently. There are still difficulties, but with continually working together and modern techniques, cotton crops may be more climate-resilient. Even in difficult areas, this method could help cotton grow in a way that is more environmentally friendly.

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Code availability

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