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Review Article

Impact of heat stress, molecular responses, and breeding strategies for heat tolerance in cotton (*Gossypium hirsutum* L.)

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ABSTRACT

Cotton is a vital cash crop in many countries due to its multipurpose usage and value addition to Gross Domestic Production (GDP), but due to climate change, it is extremely vulnerable to biotic and abiotic stresses. Among abiotic stresses, heat stress (HS) has emerged as a critical environmental constraint affecting cotton productivity and fiber quality, particularly under the escalating influence of global climate change. HS affects the cotton plant at morphological, physiological and molecular levels such as activation of various microRNAs (miRNAs), reactive oxygen species (ROS), signaling pathways including calcium, kinases, carbohydrates, gene expression regulation, epigenetic regulation, and plant hormones that help mitigate cellular damage. The purpose of this manuscript is to study the impacts of HS at critical stages of plant growth by thorough understanding of underlying mechanisms and regulatory pathways and explore the genetic underpinnings of cotton to aid breeders in employing various strategies such as conventional breeding, molecular and transgenic approaches to enhance thermal resilience. A deeper understanding of these adaptive responses is essential for sustaining cotton production in increasingly warmer climates.

Keywords: Cotton; high temperature; impact; mechanisms; strategies.

INTRODUCTION

Gossypium hirsutum L. is considered as cash crop with an estimated annual economic impact valued at $600 billion globally and continues to be the leading natural fiber produced and traded worldwide (Saini et al., 2023). The cotton sector operates across nearly 150 countries and supports the livelihoods of around 100 million families (Ashraf et al., 2024). It contributes about 0.6% of GDP and 3.1% in agriculture value addition of Pakistan. Cotton farming provides employment to millions of people in rural areas, especially in developing countries. From farming to ginning, spinning, weaving, and garment manufacturing, many sectors depend on cotton. Pakistan has a robust textile industry that relies heavily on cotton. The country is one of the largest producers and exporters of cotton textiles, including yarn, fabric, and finished garments. This sector is a major contributor to the national economy, generating significant foreign exchange earnings and providing numerous jobs. Cotton seed oil is the major source of edible oil and about 70% of edible oil requirement is met by cotton in Pakistan. Its cake is good for animal feed (Ijaz et al., 2024). There are many biotic and abiotic stresses that affect cotton production such as heat, drought, salinity, insect pests and diseases. One of the major factors that affect growth and development in cotton is high temperature (Majeed et al., 2021). It is the main aspect that limits adaptability and crop productivity, particularly at the

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critical stages of plant growth (Hatfield and Prueger, 2015; Fahad et al., 2017; Abro et al., 2023). Plant growth is affected by environmental fluctuations in temperature and each plant species has an optimum temperature range for growth.

Cotton is grown in warm climate but it is badly affected by elevated temperatures. Optimum temperature for this plant is approximately 32°C to 35°C and is affected as temperature rises above 38°C. Studies reported that by the twenty-first century, the average global temperature is expected to increase by 1.5°C (Saini et al., 2023). Heat stress (HS) is one of the biggest challenges to food security worldwide. Studies show that for every 1°C rise in temperature during the growing season, major crops yield can drop by more than 17% (Luqman et al., 2025). USA, China, India, Pakistan, Brazil, Turkey, and Australia that are responsible for approximately 75% of the global production area, currently have the capacity to achieve above-average cotton yields even at moderately elevated temperatures (Luqman et al., 2025). But, if average temperature remains increase at current pace, major producers are also likely to experience significant yield reductions. In particular, regions already cultivating cotton under conditions approaching 40°C, could face severe climate-induced stress, resulting in substantial declines in yield per unit area (Yousaf et al., 2023).

HS causes reduction in yield by affecting various morphological (Fahad et al., 2017), physiological and biochemical processes (Singh et al., 2018). Reproductive stage is mainly affected by high day temperature, leading to decreased seed number and fruit retention. However, high temperature during night causes increase in respiration rate, hinders carbohydrate supply from source to sink resulting in less fibers per seed. Capability of boll retention is decreased due to which abscission of cotton bolls reaches up to 40% (Majeed et al., 2021). Crop water requirement is also increased under HS because there is greater evapotranspiration at elevated temperature (Sadok et al., 2021).

Generally during reproductive phase, HS has negative effects on pollen viability, fertilization and grain formation. Pollination stage is badly affected by HS in different crops. Grain yield in major cereal crops decreases due to increase in temperature beyond the optimum level. If there is increase in 1°C rise in temperature above the optimum growth range, cotton lint yield decreases by upto 110kg/ha (Ijaz et al., 2024).

This review brings together cutting-edge approaches including CRISPR/Cas, genome-wide association studies (GWAS) along with traditional breeding approaches to accelerate the breeding of cotton varieties better equipped to withstand HS. The goal is to provide comprehensive knowledge of future breeding initiatives by highlighting key strategies that strengthen cotton’s resilience, helping ensure sustainable yields in an increasingly warmer world.

IMPACT OF HS IN COTTON

Morphological traits

Seed germination is affected by high temperatures above 38℃ which leads to reduction in germination, showing maximum seedling growth at 30℃ (Abro et al., 2023). Heat stress (HS) has a strong negative effect on cotton roots, causing root length to shrink by 34% to 67% (Parkash et al., 2024) and total root biomass to drop by 41% to 77% depending on how extreme the temperature is (Wang et al., 2024a). It hampers growth of root hairs, thereby reducing water and nutrients uptake (Yousaf et al., 2023; Shifa et al., 2024), hence, many physiological processes are disturbed and results in reduced yield (Zahid et al., 2016). HS also modifies the composition and structure of the cell wall, weakening the mechanical strength and reducing the roots' ability to adapt to stress. Optimum temperature for root growth is 22℃/30°C and distribution of roots is distressed above 32°C/40°C (Luqman et al., 2025). A well-developed root system substantially improves cotton yield, even under suboptimal moisture. However, to date, only a limited number of studies have documented the genes such as *GhWRKY41* (Adjibolosoo et al., 2024), *GhROD1* (Ding et al., 2024), *GhADF1* (Qin et al., 2022), *GhARF1* (Wang et al., 2024b), *GhNAC1* (Sivakumar et al., 2021), *GhHSP17.3* (Lv et al., 2024), *GhANN11* (Luo et al., 2024), *GhZFP1* (He et al., 2019) linked with root traits in cotton under HS. The differential expression of these genes under HS has confirmed the presence of resistance in various cultivars (Ding et al., 2024).

Monopodial and sympodial branches are affected by high temperature, in a way that there are more monopodial branches and less sympodial branches for long period exposure to high temperature >42ºC (Majeed et al., 2021; Abro et al., 2023). Plant height and nodes per plant are reduced under stress, as chlorophyll contents and photosynthesis is decreased (Saleem et al., 2018). A reduction of about 33% to 46% in all these traits was detected, underscoring harmful effects of HS on cotton morphology (Yousaf et al., 2023). Leaf growth is very much sensitive to temperature and leaf angle is modified under HS (Abro et al., 2023). It significantly impairs leaf morphology such as reductions in leaf length, width, length-to-width ratio, and overall leaf area, highly influenced by both the duration and intensity of heat exposure. These traits experience greater deterioration under prolonged and severe HS (Yousaf et al., 2023).

Researchers have found that genes like *HSP70*, *HSP101*, *HsfA1*, and *HsfB1* are linked to important leaf traits and play a key role for HS tolerance in cotton (Luqman et al., 2025).

Cotton is affected negatively by HS, at square formation, bud initiation and flowering stage, which is the most sensitive stage to HS (Farooq et al., 2015; Kamal et al., 2017a; Majeed et al., 2021). Shedding of squares and flowers is started, as temperature rises >30°C. HS is strongly linked with infertility, reduces boll retention and number of productive bolls. Poor boll development occurs due to which, boll size and boll weight are reduced because maturation phase of boll is less at higher temperatures above 40°C (Kamal et al., 2017b; Singh et al., 2018). Best approach to enhance yield is to reduce shedding of flowers and bolls (Tariq et al., 2017).

Physiological traits

Physiological traits are greatly affected by HS and hence reduce yield. In cotton, optimum photosynthesis is reported at 28°C and high temperature greater than 32°C leads to reduction in the photosynthetic activity, ultimately decreases accumulation of sucrose (Zahid et al., 2016; Abro et al., 2023). Photosynthesis is diminished or suppressed because ionic conductivity of thylakoids is increased and leads to the decreased activity of Rubisco, a key enzyme involved in catalyzing carbon fixation during photosynthesis, whose activity is impaired under stress conditions due to the inhibition of Rubisco activase (Luqman et al., 2025). According to Rehman et al. (2021), enhanced activity of *GhiHSF14* and the reduced activity of *GhiHSF21* in cotton under HS were associated with enhanced photosynthetic efficiency, thereby contributing to improved heat tolerance. It was studied that as compared to optimum conditions, high temperature above 42°C reduces photosynthesis in cotton by 30%, electron transport by 12%, and cell membrane integrity by 23% (Saini et al., 2023). Subtending leaves in cotton are the key source of carbohydrates for fruit formation. Under HS, lower fertilization rate is associated with reduced chlorophyll contents in subtending leaves. In another study, it was found that photosynthesis in subtending leaves was decreased by 39.5% in thermosensitive genotypes while, sustained in thermotolerant genotypes (Saini et al., 2023).

Chlorophyll contents are reduced under such condition due to which photosynthesis is affected negatively (Karademir et al., 2018). Cell membrane structure is disintegrated because reactive oxygen species (ROS) act as signal and induce the production of malondialdehyde through lipid peroxidation (Sable et al., 2018; Majeed et al., 2021). Protein denaturation leads to disturbance in sturdiness of cell membrane (Majeed et al., 2019b). Falling of leaves is started at earlier stages due to deterioration of proteins. As a result, cell elongation and differentiation is changed and distorts the structure of cytoskeleton and microtubules. In cotton, positive correlation is found between cell membrane thermostability and heat tolerance (Majeed et al., 2021).

Rate of transpiration is high at high temperature because conductivity of stomata increases (Abro et al., 2023). The inactivation of Rubisco due to HS leads to the reduction of stomatal permeability, as stomata control both water loss through evaporation and the exchange of CO_2, they play a vital role in the development of heat tolerant plant varieties. Enhanced conductivity of stomata provides cooling effect to leaves, a source of tolerance to heat (Luqman et al., 2025). These findings emphasize the essential role of stomatal regulation and photosynthetic efficiency for improving crop varieties that can withstand HS. Fiber quality is badly affected by inhibition of cellulose synthesis due to decreased accumulation of sucrose.

Reproductive traits

Development of male and female sexual organs (stamen and pistil) in cotton plant is badly affected by HS and causes various irregularities in flower development including discordant development of male and female organs, shorter filament, poor anther dehiscence, pollen tube growth, reduction in pollen germination and fertilization (Echer et al., 2014; Ekinci et al., 2017; Majeed et al., 2021; Abro et al., 2023; Luqman et al., 2025). It leads to the decreased pollen viability and premature abortion of reproductive organs that leads to male sterility. Pollen grain development depends on the metabolism of fatty acids, extremely sensitive to HS (Saini et al., 2023). Maximum pollen germination was detected at 28ºC, decreased at 37ºC and ceased at 47ºC (Luqman et al., 2025). Maximuum pollen tube length was recorded at 32ºC, declined significantly >32ºC and almost terminates at 44ºC (Luqman et al., 2025). Among reproductive traits, pollen tube development is highly sensitive to elevated temperatures and maximum growth is recorded at 28-32ºC, decreases above 34ºC (Zahid et al., 2016). One of the main reasons of poor pollen germination and fertilization is the reduction in carbohydrate concentration and supply, which is due to decreased photosynthesis under HS because high amount of energy is needed by pollens and pollen tubes (Saini et al., 2023; Luqman et al., 2025). Quantity of pollens is an important factor for the determination of time required by the pollen tube to access the ovule. Pollen tubes from unlimited quantity of pollens require less time and those developed from limited amount of pollens require more time to reach the egg cell. Due to limited amount of pollens, the slow growth rate of pollen tube is attributed by the disruption of the physiological connection between pollens and stigma (Zahid et al., 2016; Li et al., 2023b). The tapetum, which is inner layer of anthers, is responsible for the production of microspores and enzymes for the ejection of microspores from anther lobes. This layer is degenerated by programmed cell death induced by HS and leads to infertility.

To study the mechanism of poor pollination and fertilization during HS, the role of *GhCKI* gene needs to be studied that is upregulated in cotton. Transcription of *GhCKI* is high in the generative tissues particularly in mature anthers during stress. The *GhCKI* gene is induced in anthers of HS sensitive genotypes, could alleviate sterility under HS (Luqman et al., 2025). *GhBEE1* (Chen et al., 2017), *ARFs* (Ding et al., 2017), *GSTU24* (Chen et al., 2018), and *MPS1* (Chen et al., 2018) genes control anther indehiscence. Such genes are pivotal under stress and we need to identify HS tolerant genes for the improvement of cotton genotypes. Enzymes accountable for carbohydrate metabolism and its translocation are mainly essential under HS. These enzymes may act as marker for the loss of viability of pollen. It has reported that anther indehiscence causes male sterility in *Gossypium*. Studies revealed that *GHCKI* protein, once accumulated, ceases tissue degeneration, which can cause indehiscence of anther (Zahid et al., 2016).

Yield and quality

Fiber is the main product that is obtained from cotton and is the major objective of the producers. The yield of a plant is a complex trait and is influenced by environmental factors. HS causes reduction in seed yield, lint yield, size of bolls, boll weight, boll retention, seeds per boll, seed size and fibers per seed because rate of photosynthesis is decreased which leads to less production of carbohydrates (Majeed et al., 2021; Saini et al., 2023). Important yield parameters in cotton are boll size and number of bolls. Seed number is associated with number of locules in a boll and number of ovules in a locule (Majeed et al., 2021). Seeds per boll is basic trait that contributes to cotton yield. There are different aspects that are associated with the variability in the seeds number per boll such as lack of fertilization, restriction of embryo growth after fertilization, genotype and environment. According to Zhang et al. (2024), HS causes a noticeable drop in both boll weight and bolls per plant, mainly due to reduced pollen fertility. This decline in fertility was linked to problems in energy production and imbalanced sugar levels in the anthers. However, promoting the breakdown of sucrose could help improve pollen fertility in these conditions, which might be achieved by reducing the activity of the *GhSWEET55* and *GhSUT3A/D* genes.

Fiber development is also affected by HS. For proper fiber development, an average temperature 26ºC is considered important while temperature above 35ºC affects various fiber quality parameters such as length, strength, micronaire, uniformity, maturity and lint percent. There is reduction in fiber length and fiber strength due to HS. Micronaire value of fiber is enhanced due to which coarse fibers are formed with reduced strength (Manan et al., 2022).

Table 1. Impact of HS in cotton.

Traits	Impact of HS	References
Morphological traits	Reduction in seed germination, seedling emergence and plant height	(Abro et al., 2023)
	Increase in monopodial branches and decrease in sympodial branches	(Saleem et al., 2018)
	Leaf growth is affected and leaf angle is modified	(Abro et al., 2023)
	Bud initiation stage is affected	(Kamal et al., 2017a)
Physiological traits	Reduction in photosynthetic activity and chlorophyll contents	(Karademir et al., 2018; Abro et al., 2023)
	Cell membrane structure is disintegrated due to lipid peroxidation	(Sable et al., 2018)
	Protein denaturation	(Majeed et al., 2019b)
	Enhanced transpiration rate	(Abro et al., 2023)
Reproductive & Quality traits	Decreased pollen viability, pollination, and subsequent fertilization	(Ekinci et al., 2017; Luqman et al., 2025)
	Tapetum is degenerated and leads to male sterility	(Majeed et al., 2021)
	Flower development is adversely affected	(Echer et al., 2014)
	Shedding of squares and bolls	(Luqman et al., 2025)

	Reduction of seed size, seeds per boll, boll size, boll weight, and number of fibers per seed	(Kamal et al., 2017b; Singh et al., 2018; Majeed et al., 2021; Abro et al., 2023; Luqman et al., 2025)
	Reduction in fiber length and fiber strength	(Manan et al., 2022)
	Enhanced micronaire value	(Abro et al., 2023)

Cotton fiber mainly contains cellulose, and cellulose production is optimal within a temperature range of 25ºC to 30ºC. Under HS, cellulose synthesis is declined due to the reduced activity of sucrose metabolism enzymes (as sucrose is the major constituent of cellulose) and transformation of UDP-glucose to callose rather than cellulose. Reduction in fiber sucrose content is due to the down-regulation of sucrose transporter gene, *GhSUT-1* (Saini et al., 2023). Boll maturation period is shortened and short fibers are formed. As a result of prolonged heat, fiber quality is badly reduced due to irreparable loss of cellulose (Bo et al., 2017, Manan et al., 2022). It was studied that under HS, heat tolerant genotypes depict yield stability and produce good quality fiber (Manan et al., 2022).

MECHANISMS OF HEAT TOLERANCE

Role of heat shock proteins (HSPs)

Under HS, certain proteins accumulate in plants to sustain them from heat shock and are known as HSPs. They play an important role for the stability of cell functions and sustain the solidarity of cell. Under stress, a signal from the defense system activates HSPs coding genes, due to which accumulation of HSPs and molecular chaperons is started for the protection of proteins and cell membrane integrity (Sarwar et al., 2018). HSPs act as molecular chaperones, help to stabilize and refold damaged proteins that can become misfolded or denatured under high temperatures. This process prevents the accumulation of damaged proteins, which could otherwise lead to cellular dysfunction. HSPs contribute to increased heat tolerance by enhancing the plant's ability to manage oxidative stress and repair cellular damage. HSPs are involved in regulating various stress response pathways, including those related to cellular signaling and stress adaptation. They help the plant adjust its metabolic processes to cope with the adverse effects of HS. The heat shock response (HSR) refers to the transcriptional regulation of HSPs, triggered by HS. This response is controlled by heat shock transcription factors (HSFs) linked with HSP genes (Hao and He, 2024).

These molecules have been classified into five categories on the basis of molecular weight i.e. small HSPs (HSP20), HSP60, HSP70, HSP90 and HSP100 (Abro et al., 2023). It has been found that expression of small HSPs in plants is associated with HS tolerance. A small HSP gene (*GHSP26*) is transcribed in cotton under high temperature and drought, due to which leaves contain greater concentration of proteins that helps to sustain good yields under HS (Majeed et al., 2021). HSP60, helps in the folding and unfolding of proteins in mitochondria, also assist photosynthesis related protein, Rubisco (Luqman et al., 2025).

The up-regulation of HSP70 in plants reveals HS tolerance. Fiber development in cotton is strongly linked to *HSP70* genes and elongation of fibers is distressed due to down-regulation of these genes (Sable et al., 2018). The suppression in expression of these genes leads to the oxidative stress by enhancing H_2O_2, degrading the epidermal layer of ovule (Luqman et al., 2025). HSP90 are distinguished from other molecular chaperones due to their principal involvement in signal transduction (Lubkowska et al., 2021). Furthermore, they facilitate protein folding and are considered among the most prevalent proteins. HSP90 proteins frequently collaborate with HSP70 and their expression levels markedly increase in response to HS. HSP100 is categorized within the AAA ATPase family and executes numerous functions, including the unfolding and degradation of proteins (Lubkowska et al., 2021). In addition to enhancing plant resilience to HS, HSP100 is integral to fundamental cellular processes, such as the development of chloroplasts (Mishra and Grover, 2016; Majeed et al., 2021). Role of various HSPs during HS is depicted in Figure 1.

Role of microRNAs under heat stress

HS causes intensive reprogramming in gene expression events. Key gene regulators are transcriptional elements that induce expression of many genes during stress and can cope with undesirable environmental condition. It has been found that microRNA (miRNA) perform significant role at molecular level under HS. Different regulatory functions of miRNAs in plants have been exposed through technology advancement. miRNAs are called "killer RNAs" which are categorized as non-coding small RNAs (ncRNAs). miRNA is composed of 21 nucleotides which are hairpin precursors of single-stranded RNA. During HS in cotton, certain miRNAs are activated to cope with environmental conditions. miRNA binds with an Argonaute (AGO) protein to form a complex called RISC (RNA-induced silencing complex). This complex then attaches to complementary sequences on target mRNA, leading to either the degeneration of the mRNA or translational suppression, regulating expression of genes at post-transcriptional level (Luqman et al., 2025). In addition, miRNAs help regulate the levels of HSPs during HS by associating with 3′ untranslated region (UTR) of the mRNAs that code for these proteins. This interaction can either boost the production of HSPs or protect their mRNAs from being broken down. As a result, miRNAs help sustaining the cell’s response to heat, ensuring that HSP levels are properly balanced to maintain cellular stability and prevent proteins from misfolding in stressful conditions (Islam et al., 2024) or translational suppression, regulating expression of genes at post-transcriptional level (Luqman et al., 2025).

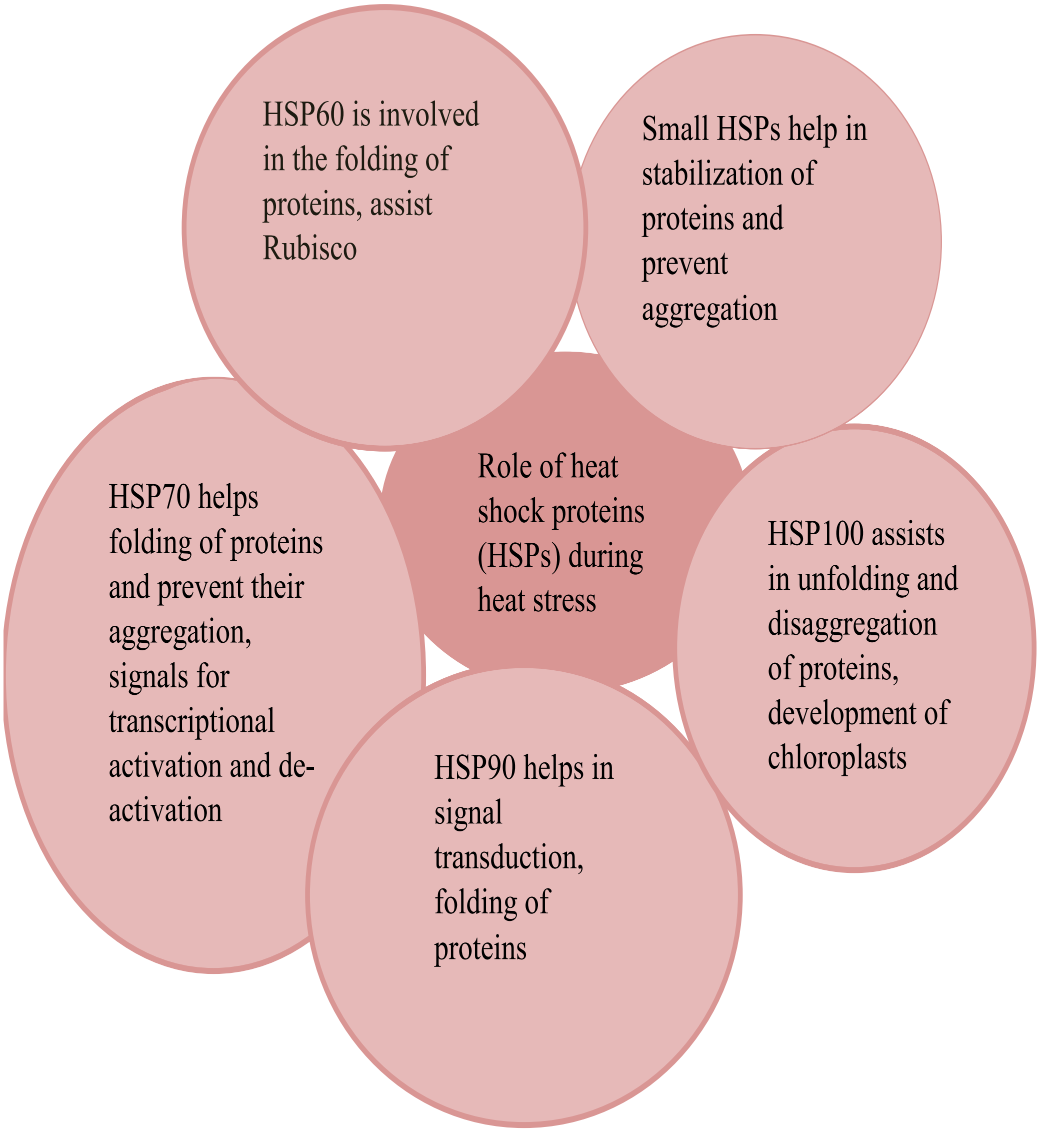

Figure 1. Role of HSPs during heat stress in cotton.

In addition, miRNAs help regulate the levels of HSPs during HS by associating with 3′ untranslated region (UTR) of the mRNAs that code for these proteins. This interaction can either boost the production of HSPs or protect their mRNAs from being broken down. As a result, miRNAs help sustaining the cell’s response to heat, ensuring that HSP levels are properly balanced to maintain cellular stability and prevent proteins from misfolding in stressful conditions (Islam et al., 2024). miRNAs help to control various stress-responsive pathways, including those related to antioxidant defense, osmotic adjustment, and protein synthesis. By regulating these pathways, miRNAs contribute to the plant’s overall ability to withstand HS. Based on the preservation traits of miRNA in cotton ovules and other processes, many families of miRNA and their target areas are studied. Currently, there are almost 89 miRNAs under *Gossypium* at Plant Micro RNA database (Zahid et al., 2016), and a set of miRNAs has been detected that are activated under HS (Zahid et al., 2016). During HS, expression of miRNAs is enhanced and target *SPL* genes which play role for flower development (Chen et al., 2019). Upregulation of miR157 stimulates *SPL* genes due to which small sized bolls with less number of seeds are formed (Liu et al., 2017). Expression of miR162 is upregulated that controls the expression of genes through zinc finger proteins (ZFPs). ZFPs play essential part in abiotic stress tolerance in cotton and are upregulated in stress condition (Majeed et al., 2021).

Plant hormone abscisic acid (ABA) is required for growth regulation and stress responses. During HS, ABA controls the growth of plant through its production, regulation and signal transduction, thereby regulating ROS levels. ABA, particularly, controls HS transcription factors (HSFs) and HSPs, further assisting plants to cope with HS (Luqman et al., 2025). ABA triggers the accumulation of miRNA159, that cleaves other transcripts to upregulate the *MYB33* and *MYB101*. There are different specifications of miRNA up-regulation and down-regulation in response to ABA in different crops (Zahid et al., 2016). Understanding the role of miRNAs in HS responses, can lead to the development of genetically modified or selectively bred cotton varieties with enhanced heat tolerance. This is achieved by manipulating miRNA expression to boost the plant's resilience.

Role of antioxidants against oxidative stress

In plants due to HS, various changes are induced in metabolic processes due to which, reactive oxygen species (ROS) are accumulated in cells. When there is high concentration of ROS than plant's capability to scavenge, leakage of ROS is started into other plant organs, which is known as oxidative stress (Qamer et al., 2021). Oxidative damage is caused to important cell organelles and molecules like DNA, lipids and proteins. ROS comprise of free radicals (OH, O^{2}-), and non-radicals ($^1O_2$, H_2O_2). In cotton during HS, high concentration of H_2O_2 is negatively associated with seed cotton yield (Saini et al., 2023).

Though, there are many devastative effects of ROS on plant processes but they act like signals for HSPs for heat tolerance mechanism in plants. However, a stable concentration of ROS maintains activities such as apoptosis, antimicrobial phagocytosis, decontamination of harmful constituents and various signaling pathways (Considine et al., 2015; Singh et al., 2016; Luqman et al., 2025). Genes coding for osmoprotectants, regulatory proteins and detoxifying enzymes are up-regulated. Heat tolerant genotypes in cotton could be assessed by the concentration of ROS, contributes to an important physiological trait as selection criteria (Majeed et al., 2019b). Genotypes with less concentration of ROS and enhanced concentration of antioxidants refers to HS tolerant under elevated temperatures. Although ROS are produced in many cell organelles, but the main sites are photosystem I (PSI) and photosystem II (PSII) in chloroplasts, interrupt electron transport. These photosystems are damaged by HS, and therefore, photosynthetic activity is altered under such prevailing condition. Hydroxyl radicals react with different biomolecules and cause oxidative damage to DNA by affecting nucleic acids, protein oxidation leading to enzyme inhibition, lipids by lipid peroxidation, and almost all components of cell that stimulates programmed cell death (PCD). Peroxidation of lipids in cell membrane affect its permeability and its function. These biomolecules are also oxidized by singlet oxygen (Majeed et al., 2021). It was studied that there is high concentration of ROS in cotton at reproductive stage due to enhanced lipid peroxidation by malondialdehyde (MDA), affecting various cell organelles (Qamer et al., 2021).

To alleviate the harmful effects of ROS, cotton plants start to synthesize different enzymatic antioxidants e.g. superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) as well as non-enzymatic antioxidants (carotenoids, flavonoids, tocopherols, ascorbate) that play their role as detoxifying agents (Qamar et al., 2021). It was reported that increased activity of SOD, CAT and POD leads to lower concentration of H^2O^2, linked with HS tolerance in cotton (Yousaf et al., 2023).

Signaling pathways during heat stress

Signal transduction in plants is an intricate mechanism because it includes many components. Primary cell organelle that is most sensitive to HS is plasma membrane, perceives stress through different strategies. RNA sequencing and GWAS shows that genes up-regulated by HS in cotton induce ABA, mitogen-activated protein kinases (MAPKs) and the calcium transduction pathways. These strategies include the opening of calcium gates leading the calcium influx, and may activate various signaling pathways. Main signaling pathways are calcium, kinases, ROS, carbohydrate, transcriptional factors (TFs) and plant hormones. As a source of signal transduction, calcium is capable to produce resistance to several abiotic stress factors. Triggered calcium signaling (TCS) induces binding of calcium to calmodulin (CaM), which plays significant role in heat shock signal transduction pathway. TCS activates calcium-dependent protein kinases (CDPKs) that stimulates other proteins by adding phosphate groups. Calcium signals also activate phosphatidylinositol-4-phosphate 5-kinases (PIPKs) that stimulates lipid signaling. These play a significant part in the tolerance mechanisms in plants. All these molecules and signal transduction pathways enhance HS response, leading to programmed cell death (Zahid et al., 2016).

In response to HS, MAPK pathways get activated and regulate various stress related genes and proteins. This pathway helps in coordinating the plant's overall response to HS, including adjustments in gene expression and protein activity. Carbohydrates play significant part in HS response by mediating energy metabolites. Transcriptional factors (TFs), during HS in cotton, induce various plant hormones [such as indole acetic acid (IAA), abscisic acid (ABA), salicylic acid (SA), ethylene (ET), jasmonates (JA) and brassinosteroids (BR)] signaling pathways, some TFs play major part in upregulation of genes to regulate the pathway (Zahid et al., 2016).

Epigenetic regulation

Epigenetics refers to changes in the chromatin structure of the stress associated genes at both transcriptional and post-transcriptional levels through different chemical alterations, which influence how genes are turned on or off. DNA methylation (DM) represents complex biochemical process that necessitates the participation of diverse enzymes and cofactors. The process initiates when DNA methyl transferase recognizes a CpG dinucleotide (Ravi et al., 2025). This epigenetic modification exerts an influence on chromatin structure, ultimately resulting in the repression of gene transcription, as DM is pivotal in modulating gene expression throughout plant development and response to stresses (Luqman et al., 2025). Methylation of the cytosine leads to the production of 5-methylcytosine (5mC), exhibits crucial role in genome stability, gene silencing, developmental stages, and mediating responses to HS (Luqman et al., 2025). Through both mechanisms, methylation and demethylation, 5mC suppresses the gene transcription, thereby assisting plant to withstand HS (Lawson et al., 2025).

Alterations in histone proteins take place through histone methylation, phosphorylation, acetylation, and ubiquitination at various amino acids. These alterations in histone proteins in cotton during HS play significant part in the regulation of genes. HS causes changes in histone modifications, making certain stress-responsive genes more accessible and increasing their expression such as the methylation of histone H3 at various amino acids (H3K4me, H3K36me) leads to up-regulation of genes, while methylation of histone H3 (H3K9me, H3K27me) causes down-regulation of genes. Alteration in histone proteins is essential in the development of cotton anthers (Ijaz et al., 2024). Research related to epigenetic regulation in cotton under HS have been summarized in Table (2). Under HS, cotton plants undergo DNA methylation to turn on or off specific genes that help in stress response such as genes involved in HSP production are activated, while transcription of three genes in cotton anthers (including 2-jumonji C and a histone mono-ubiquitination) is suppressed (Zahid et al., 2016).

Table 2. Epigenetic regulation in cotton under heat stress

Species	Tissue	Heat treatment	Epigenetic marks	Associated gene	Modification	Impact	References
G. hirsutum	Anther	38-40˚C (DT), 28-31˚C (NT)	Histone modification	*GhAOS, GhJAZ1, GhAOC2*	*H3K4me3, H3K27me3*	Male sterility	Li et al. (2023a)
G. hirsutum		36.5 ˚C (DT), 28 ˚C (NT)	DNA-methylation	*GhNDUS7, GhCOX6A, GhATPBM, GhCX5B2*	*CG,CHG, CHH-methylation*	Anther developmnt	Zhang et al. (2020)
G. hirsutum		39-41˚C (DT), 29-31˚C (NT)	DNA Methylation	*DRM2/CMT2*	*CHH-methylation*	Male sterility	Ma et al. (2018a)
G. hirsutum		35-39˚C (DT), 29-31˚C (NT)	DNA Methylation, Histone modification	*DRM1, DRM3, NERD, SAHH1, HAT1, HDA15, HDA2C*	--	Anther development	Min et al. (2014)

DT: Day Temperature, NT: Night Temperature

There are certain limitations for the application of epigenetic modifications for enhanced HS tolerance in cotton and other plants. One major challenge is the intricacy and instability of these modifications. Changes like DNA methylation and histone modifications are often rescindable, which makes it difficult to preserve these alterations consistently over time and through generations, limiting their practical use in crop breeding (Manav et al., 2024).

BREEDING APPROACHES TO TOLERATE HEAT STRESS

Conventional breeding strategies

Conventional breeding is the most commonly used approach for the development of heat tolerant cotton genotypes. It includes direct selection or selection after hybridization. For this purpose, screening of germplasm is the basic step to find the genetic variation and best performing genotypes for various morphological, physiological and reproductive traits under HS. Screening of genotypes under stress condition provides information about those genotypes and traits which perform better and produce good yield as compared to stress susceptible genotypes, and could be selected for improving the existing varieties and future crop breeding (Fahad et al., 2017; Ali et al., 2023). Several studies have been documented for HS tolerant genotypes from the gene pool (Abro et al., 2015; Abro et al., 2022; Zafar et al., 2022). Exotic, wild and distant lines (*G. arboreum, G. herbaceum,* and *G. barbadense*) exhibit potential for their utilization in plant breeding because of their broad genetic base and distinct traits associated with HS tolerance (Luqman et al., 2025). However, there are many factors such as hybrid sterility and agronomic issues, linked with gene transfer (Mammadov et al., 2018).

Screening of genotypes is carried out both in controlled environment and field. Screening in field is more useful as it provides natural environment to plant for growth and reproduction and fulfills the requirement of breeders and growers. After identification of suitable trait, next step is to transfer it to the desired genotype and purified through selection. For this purpose, various conventional breeding approaches that are widely used in cotton include single plant selection, bulk method, and pedigree method. These approaches combined with molecular strategies such as marker-assisted selection (MAS) for screening, and recombinant DNA technology to introduce genes across species, enable efficient genetic improvement of cotton, as conventional breeding is time taking, laborious and tedious process (Saini et al., 2023; Luqman et al., 2025). HS tolerant varieties in Pakistan have been summarized in table (3).

Table 3. HS tolerant varieties developed by conventional breeding in Pakistan.

Varieties	Developed by	References
MNH-552	CRI, Multan	Rahman et al., 2004
CRIS-19	CCRI, Sakrand	Rahman et al., 2004
CIM-608	CCRI, Multan	Anjum et al., 2014
CRIS-129	CCRI, Sakrand	Ahsan et al., 2017
RH-662	CRI, Khanpur	Yasin et al., 2019
RH-668	CRI, Khanpur	Yasin et al., 2020
MNH-886	CRI, Multan	Anwar et al., 2022
VH-260	Cotton Research Station, Vehari	Anwar et al., 2022
VH-259	Cotton Research Station, Vehari	Anwar et al., 2022
VH-189	Cotton Research Station, Vehari	Anwar et al., 2022
MNH-1035	CRI, Multan	Anwar et al., 2022
RH-647	CRI, Khanpur	Shaheen et al., 2021
VH-305	Cotton Research Station, Vehari	Saleem et al., 2021
Cyto-177	CCRI, Multan	Saleem et al., 2021
Cyto-515	CCRI, Multan	Saleem et al., 2021
CIM-600	CCRI, Multan	Ali et al., 2022
CIM-616	CCRI, Multan	Ali et al., 2022
CIM-482	CCRI, Multan	Ali et al., 2022
CIM-446	CCRI, Multan	Ali et al., 2022
FH-142	Cotton Research Station, Faisalabad	Ali et al., 2022
BS-15	Bandesha Seed Corporation	Ali et al., 2022

MNH-992	CRI, Multan	Ali et al., 2022
FH-Lalazar	Cotton Research Station, Faisalabad	Sarwar et al., 2023
FH-152	Cotton Research Station, Faisalabad	Sarwar et al., 2023
FH-444	Cotton Research Station, Faisalabad	Sarwar et al., 2023

CCRI. Central Cotton Research Institute, CRI: Cotton Research Institute.

Molecular and biotechnological approaches

Molecular markers and various biotechnological advances play significant role in improving stress tolerance in cotton (Mubarik et al., 2020). Advanced breeding approaches along with quantitative trait loci (QTL) mapping are valuable to study HS tolerance. QTL mapping, facilitates marker-assisted breeding, is an effective tool to study genetic basis of desired traits and locates loci on the chromosomes linked with phenotype of an organism. 138 QTLs for eight heat related traits have been recognized including lint yield, cotton seed yield, and quality (Saini et al., 2023).

Various studies identified QTLs linked to heat tolerance using segregating populations derived from crosses between heat-tolerant and heat-sensitive cotton varieties. These studies focused on traits like flowering time, boll development, and fiber quality under HS. Specific QTLs associated with heat tolerance have been found on several chromosomes in cotton. For example, QTLs linked with boll retention, yield, and fiber quality under high temperatures have been mapped at chromosomes 5, 7, and 11 in various studies. Advances in genomics and breeding technologies have allowed for finer resolution mapping of QTLs. Researchers have used single nucleotide polymorphism (SNP) and next-generation sequencing to identify QTLs with greater precision and to dissect complex traits related to heat tolerance. Some QTLs identified in initial studies have been validated in different cotton varieties and environmental conditions, confirming their role in heat tolerance (Ijaz et al., 2024). Various studies related to QTLs identified for heat tolerance in cotton have been summarized in table (4).

MAS is an indirect selection procedure, preferably used because it takes less time and produce accurate results. It is an efficient strategy to accelerate the breeding programs involving development of abiotic and biotic stress tolerant varieties. A variety of molecular markers, including Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), and Random Amplified Polymorphic DNA (RAPD), have been employed across diverse crops for the detection of QTLs linked with abiotic stresses (Majeed et al., 2019a). Nevertheless, Single Nucleotide Polymorphisms (SNPs) and Simple Sequence Repeats (SSR) represent the most prevalent markers employed for the detection of QTLs, correlated with HS and other forms of abiotic stress in plants. According to Rani et al. (2022), 26 linkage groups in conjunction with 175 QTLs were detected within cotton genotypes that exhibit both heat resistance and susceptibility. Furthermore, 17 QTLs were documented linked with 23 distinct morpho-physiological traits related to HS resistance in cotton. The traits associated with the studied QTLs not only facilitated the identification of HS resistant lines but also elucidated the most critical traits to concentrate on during the development of heat-resistant cotton cultivars.

Genomic selection (GS) with marker assisted selection is another efficient strategy, that uses genome-wide markers that have collective effect on a trait and helps in choosing promising genes. It helps in rapid variety development by shortening period through effective progeny selection in early generations, and studies effects of heat at various levels in cotton (Saini et al., 2023). GWAS is a scientific approach that studies genotypic and phenotypic variations to detect the genetic underpinnings of the trait of interest in genomes of different populations within a species (Ahmed et al., 2024). GWAS provide an efficient substitute to the screening process of large populations. As a result, GWAS has become extensively utilized in diverse research contexts to detect QTLs associated with complex traits. Significant efforts have been directed towards elucidating the genetic architecture of phenotypic traits by examining associations between SNP markers and phenotypes. This strategy enables the detection of various QTLs through mapping, supported by linkage disequilibrium (LD) analysis (Luqman et al., 2025). These developments have substantially enhanced our comprehension of the genetic basis of various agronomic traits in crops including cotton (Su et al., 2016; Majeed et al., 2021). Using DNA sequencing, researchers can identify SNPs across different individuals or plants. By comparing their DNA sequences, it becomes possible to reveal common genetic variations present within the genome (Su et al., 2024). By using GWAS, genetic factors were identified controlling male sterility in cotton under HS (Ma et al., 2021). Moreover, the use of Genomic Prediction (GP) has enhanced selection efficiency through the reduction of

Table 4. Quantitative trait loci (QTLs) identified for heat tolerance in cotton.

Population	Chromosome	Loci	Approach	Associated trait	Reference
94 F2 progenies (*MNH-886 x MNH-814*)	2, 3, 5, 6, 15, 16, 18, 19, 23, and 26	--	Linkage mapping (CIM)	Physiological traits	Rani et al. (2022)
218 natural inbred accessions	A01, D01, D05	*A01-9098282, D01-8274957, D05-23761911*	GWAS, TWAS	Pollen viability	Ma et al. (2021)
95 RILs (TM-1 x M24016)	A01, A09, A13, D10, and D12	--	Linkage Mapping (ICIM)	Canopy traits, leaf area index	Pauli et al. (2016)
118ᵃ F2.3 progenies (s NM24016/TM1)	LG17, LG20	*S3, G3800*	Linkage mapping (CIM)	Stomatal conductivity, lint yield	Pauli et al. (2016)
RILs: MAB1 (117), MAB2 (113)	--	--	Linkage mapping (CIM)	Fiber traits	Dabbert (2014)
406 F2 (G. hirsutum cv. Siv'on × G. barbadense cv. F-177)	Chr06, LGA02, LGA05, LGD03	*pAR3-32a, pAR402b, pAR248, pGH232a*	Linkage mapping	Canopy temperature, chlorophyll contents	Saranga et al. (2004)

[a] Number of accessions, CIM: Composite Interval Mapping, TWAS: Transcriptome-wide association studies, ICIM: Inclusive Composite Interval Mapping.

time and breeding cost (Billings et al., 2022). To date, 82 GWAS studies have been documented including 72 studies in *Gossypium hirsutum*, 8 in *Gossypium arboreum* and 2 in *Gossypium barbadense* mainly for abiotic stress (salinity, high temperature, drought), yield and fiber (Yasir et al., 2022). 4,820 genes linked with 13 fiber traits were documented through GWAS, providing a substantial genetic resource for fiber quality improvement (Ma et al., 2018b). Additionally, through variations in transcriptome along with GWAS, three loci linked with HS tolerance were documented, collectively containing 75 protein-coding genes and 27 long non-coding RNAs (Ma et al., 2021). Another study carried out by Han et al. (2022), 30,089 expression quantitative trait loci (eQTLs) linked to around 10,000 genes were detected through GWAS. Among these, 19 genes linked with salt and HS tolerance were found in cotton. These genes are located on chromosomes 1, 5, 6, and 8. In addition, several other studies have documented various SNPs, alleles and genes related to important cotton traits that could help enhance the plant's resilience to different types of abiotic stress.

Several factors can hinder the effectiveness of GWAS in accurately identifying true connections between genetic variants and phenotypic traits. One key limitation is variability in phenotypic data. Prior GWAS analysis, it is critical to perform a thorough evaluation of phenotypic datasets to detect and address outliers (Xiao et al., 2022). Excess dissimilarity or uncorrected outliers can compromise the reliability of the results, potentially resulting in false positive or false negative links (Jiang et al., 2025). Therefore, any identified outliers should be carefully assessed for their potential influence on the analysis before proceeding with the GWAS.

After data clarification, emphasis should be placed on traits exhibiting moderate to high heritability, as this reflects the degree to which genetic variation contributes to phenotypic expression (Gesteiro et al., 2025). Additionally, population size plays a pivotal role in the effectiveness of GWAS; larger sample sizes enhance the efficiency to detect genuine genetic associations (Tibbs Cortes et al., 2021). Population structure represents a third significant challenge in GWAS, as it influences the genetic association among individuals within population under study. Recognizing historical or ancestral relations is critical, since unequal genetic associations can compromise the validity of results. Failure to account for population structure may result in spurious genotype-phenotype correlations (Altaf et al., 2025). To address this, software such as STRUCTURE (version 2.3.4) is commonly used to infer population structure by clustering individuals into genetically distinct groups (Tian et al., 2025).

Linkage disequilibrium (LD) presents another significant challenge in GWAS, as it affects the accuracy and resolution of genetic association studies. LD refers to the non-random relationship of alleles at various loci within a population. It plays a crucial role in identifying tightly linked genetic markers, such as SNPs, help localize important genomic regions. Ignoring LD can result in the inclusion of both causal and non-causal alleles, potentially leading to misleading associations. LD analysis is essential for estimating the number of markers required to achieve adequate genome coverage. High LD suggests that fewer markers may be sufficient, but extended LD can increase the risk of false relationship, underscoring the importance of assessing LD early in the study (Luqman et al., 2025).

Transgenic approaches

For the development of HS tolerant cotton genotypes, transgenic approaches have been useful (Batcho et al., 2021; Esmaeili et al., 2021). It is known as successful transgenic crop due to the insertion of chewing insects-resistant genes. Performance of different physiological and biochemical indicators is enhanced in transgenic cotton. In recent times, HSP coding gene (*AsHSP70*) was transformed in cotton from sisal hemp (*Agave sisalana*) through *Agrobacterium*-mediated transformation (Batcho et al., 2021). Under HS, up-regulation of *AsHSP70* in cotton particularly reduces cell electrolyte leakage and improves membrane stability index. There is improved germination percentage and pollen tube development due to the up-regulation of *Arabidopsis* HSP 101 (*AtHSP101*) in transgenic cotton. This gene is not expressed generally but play an important role under HS (Majeed et al., 2021; Saini et al., 2023).

In another study carried out by Zhang et al. (2021), *SikCuZnSOD3* gene was detected, linked with salt, water deficit, and HS. Through *Agrobacterium*-mediated transformation method, this gene was transferred into cotton. The resulting transgenic cotton lines exhibited improved growth with increased levels of sugars, proline, water content, and antioxidants compared to non-transgenic plants under stress.

Lint yield is improved in transgenic cotton under HS and no negative effects have been observed in the absence of stress. It was studied that up-regulation of rice SUMO E3 Ligase gene, *OsSIZ1*, in cotton improves photosynthesis under HS and drought. Prior to HS, the photosynthetic rate in the transgenic lines was approximately 72%, while this rate enhanced to 108% under HS (Esmaeili et al., 2021).

HS tolerant genotypes could be developed through the incorporation of HS associated genes in cotton for sustainable lint yield. Another HS related gene from *Arabidopsis* (*AtSAP5*), encoding an A20/AN1 zinc finger domain-containing protein, was transformed in cotton that protects growth under elevated temperature and increases HS tolerance through enhanced transcription of stress responsive genes. Reproductive systems are greatly affected by climate change and enhanced stress tolerance of these systems will lead to improved yield in transgenic cotton (Luqman et al., 2025).

While the latest biotechnological and transgenic approaches are effective, their complex procedures and long incubation times make the transformation rate relatively low in cotton. However, genome editing approach has been very useful for tolerance against abiotic stresses including HS in cotton because this approach can be used to mutate or silence genes at gene expression or post-transcriptional levels.

CRISPR/Cas9 (Clustered regularly interspaced short palindromic repeats) has proven to be effective in model plants like Arabidopsis. However, studies have documented the targeted genome editing in cotton. CRISPR can also be used to mutate or silence several genes in a gene family (Khan et al., 2023). In fact, multiple genes such as *GhARG* and *GhCLA1* have been edited simultaneously in allotetraploid cotton (Luqman et al., 2025). However, there are still some challenges and low success rate in achieving the desired transformations. Its application is confined to cotton and requires improvement (Luqman et al., 2025).

Silencing of *PYHA1* genes in cotton through RNAi results in heat tolerance by improving the root system and increasing photosynthetic rate. *FPGS3*, *GhHS126*, *GhHS128* and *IAR3* genes have been recognized for HS tolerance in cotton, that are upregulated under HS. On the other hand, expression of genes like *CTL2*, *RPS14*, *CIPK*, *LSm8*, and *ABCC3* was decreased under HS. Therefore, using the CRISPR/Cas9 system to precisely adjust the expression of these genes, whether they are overexpressed or under expressed, offers a promising approach, assisting cotton cope with the harmful impact of HS (Luqman et al., 2025). Recent findings indicate that the *MAP3K65* gene is transcriptionally activated in response to diverse signaling molecules, pathogenic invasion, and HS. This gene appears to regulate growth and developmental pathways negatively, thereby increasing plant susceptibility to biotic and abiotic stresses. Notably, silencing of GhMAP3K65 enhances cotton's resistance to both pathogen infection and elevated temperatures. Hence, GhMAP3K65 gene appears as a viable candidate for targeted genome editing using the CRISPR-Cas9 system, with potential applications in improving HS tolerance in cotton (Zhai et al., 2017).

GhCKI plays a negative role for male fertility in cotton under HS. Male sterility of GhCKI was reported through conventional genetic modification, thereby constraining its utility in breeding strategies. Another study carried out by Li et al. (2025), CRISPR/Cpf1 and CRISPR/Cas9 systems were employed to develop weak promoter alleles of GhCKI, enabling modulation of anther HS tolerance traits. This led to the development and characterization of two novel cotton lines exhibiting enhanced HS tolerance, due to the variations in the GhCKI promoter. Further molecular analysis revealed that this increased thermotolerance is linked to impaired binding of HS-responsive factors (GhMYB4 and

GhMYB73) to the GhCKI promoter, thereby reducing its heat-induced expression. This work not only offers an effective strategy for creating useful allelic variants but also provides valuable germplasm and insights to support the breeding of heat resilient cotton cultivars.

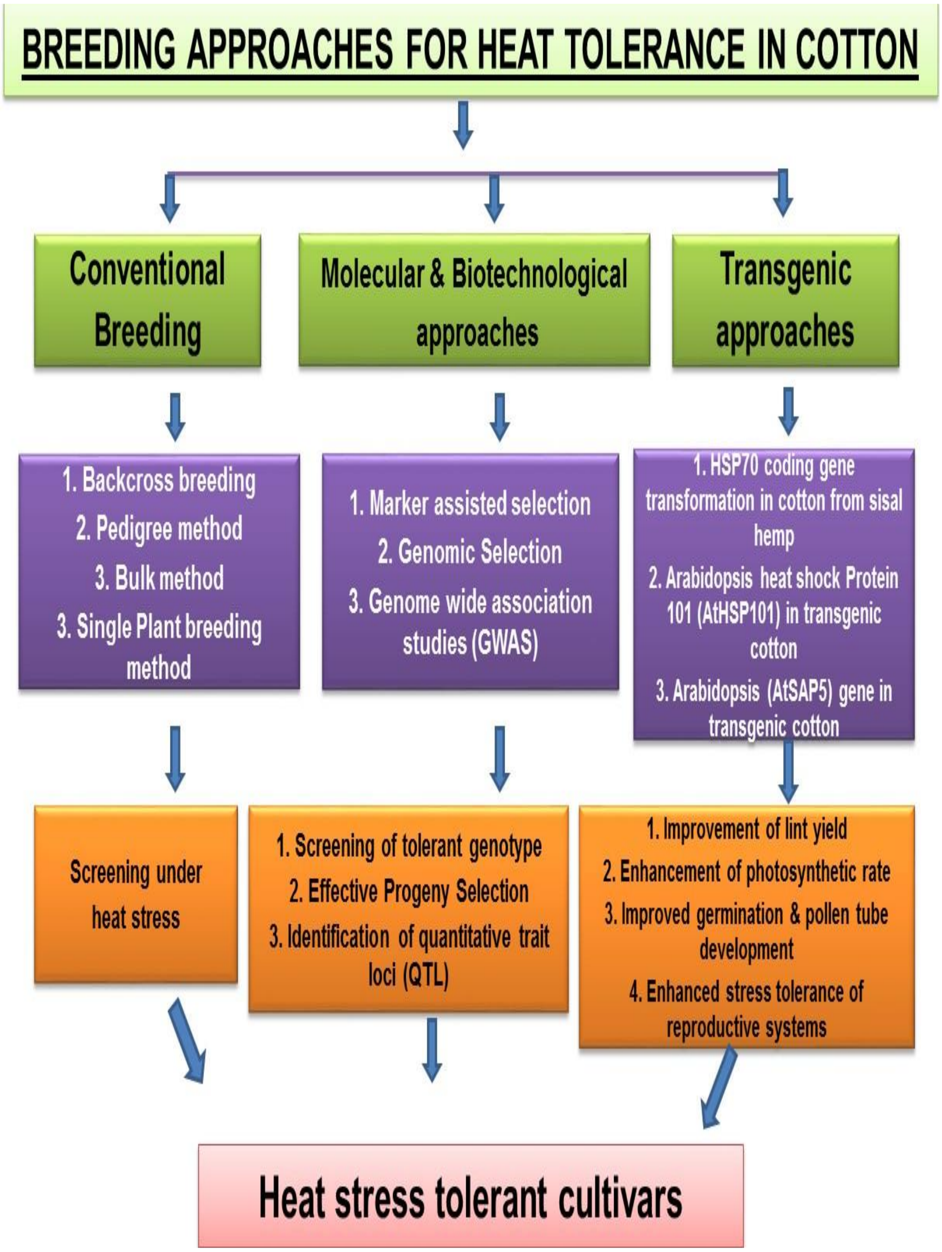

Figure 2. Breeding approaches for heat tolerance in cotton.

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CONCLUSION

All phases of growth and development in cotton are greatly affected by HS. Fiber quantity and quality is reduced because physiological and molecular processes are inhibited by elevated temperatures. HSPs coding genes and other heat responsive genes play an important role to induce stress tolerant mechanisms and proteins against stress condition. Various conventional breeding, molecular and biotechnological approaches are being used to develop heat tolerant genotypes for sustainable cotton production. Several proteins, genes and metabolites that are activated under stress condition could be used as markers for the development of HS tolerant cotton. The combination of GWAS with genomic prediction has been ascertained as a powerful tool to reveal the abiotic stress tolerance and improvement of cotton. Genome editing tools including CRISPR system has made a tremendous role through the modification of genes for stress tolerance in plant sciences. All these approaches will be helpful to cotton breeders for the development of HS tolerant and high yielding varieties in the scenario of climate change.

AUTHOR’S CONTRIBUTION

Iqra Parveen conceived the idea and compiled the manuscript. Iqra Parveen, Muhammad Safyan, Farrukh Ellahi and Wajeeha Khan contributed in collection of literature and write up, Jehanzeb Farooq and Amna Nazir reviewed and edited the paper, Ghulam Sarwar proofread the manuscript. All authors read and approved the final manuscript.

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All authors agreed to publication of manuscript in its present form.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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