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# Temperature Extremes in Cotton Production and Mitigation Strategies

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#### Abstract

Cotton is an important cash crop, providing raw material for different industries and plays crucial role in the economy of several countries. It requires optimum temperature for economic production and causes reduced yield otherwise. Extreme temperature, more importantly, high temperature causes serious yield reduction in cotton by affecting its physiology, biochemistry and quality leading to poor agronomic produce. Freezing temperature also affect the germination percentage and seedling establishment. Several breeding and genomics based studies were conducted to improve the cotton production under high and low temperature stress in cotton. Here we overviewed several agronomic practices to mitigate the effect of extreme temperature, and multiple breeding and molecular approaches to enhance the genetic potential of cotton for temperature tolerance by Marker assisted selection or transgenic approach.

Keywords: cotton, genomics, heat stress, freezing stress, marker assisted selection

# 1. Introduction

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Ever-increasing variability in world climate is threatening the cotton production globally due to temperature extremes, drought stress and irregular rainfall patterns. More than 50% yield reductions in arable crops has been accounted due to these said stresses worldwide [1]. Cotton production is severely affected due to these abiotic and several biotic stresses, thus resulting in

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reduced yields and inferior harvest quality. Having indeterminate growth habit, cotton crop bears a complex set of fruiting pattern which is considered to be severely prone to climatic interactions as well as management techniques with differential response [2]. Cotton plant responds to various stresses differently, depending upon the stress severity and the developmental stage. Among various aforementioned stresses, temperature stress in cotton is of key importance as it may cause drastic impact during germination, early growth season, flowering and during the boll formation stages. As the change in global climate is inclined to cause increase in average temperatures, therefore, high temperature may impact cotton crop in the form of longer growing seasons, more or fewer rainfalls, and thus a shorter growing period. Whereas, low temperature during the planting time impairs the seed germination process, oppositely high temperature is also an undesirable feature during planting time. Temperature stress in terms of both cold and heat stress induces a differential metabolic and physiological responses in cotton, through alterations in plant photosynthetic performance, oxidative balance, normal protein synthesis, stomatal closure, membrane damage, lipid peroxidation and carbohydrate production [3-5]. In consequence, various stress responsive mechanisms are triggered by molecular networks to stabilize the internal homeostasis by protecting and repairing of damaged membranes and proteins [6]. Meanwhile, certain heat shock proteins and antioxidant enzymes get activated to combat with induced oxidative and membrane damage within the plant body, resulting in plant tolerance to imposed stress. Still a number of key molecular and physiological mechanisms involved in this homeostasis stabilizing process are under way to find. Here, we discussed the recent advances and understandings in this regard, how the temperature stress affects cotton crop and its induced response by crop plant.

#### 2. Critical stages of cotton development for temperature stress

During late developmental stages, high temperature could lead to increased shedding of flower buds. Boll retention is utmost desirable for higher values of harvests, while high temperatures during this stage severely affects the boll retention, as compared to any other factor. Because high temperature also causes altered boll development (boll size) and maturation period [7]. Similarly, high temperature was also reported to affect the fiber quality in terms of high micronaire values and fiber strength, which are undesirable traits [7]. Low temperature stress, on the other hand, is also devastating during the germination phase of cotton crop as well as for fiber development stage by delayed elongation period and reduced cell wall thickening [8]. Therefore, the stress impact can be categorized in one way, depending upon the severity and duration of temperature stress; on the other hand, the crop growth stage under stress determines the ability of crop plant to tolerate the imposed stress. There is substantial data reporting severe yield reduction under heat stress during late reproductive stages of flowering and boll formation, thus signifying flowering stage as most critical to heat stress [9] along with stand establishment, boll formation and fiber development stages. Pollen development, pollen tube growth, and fertilization are postulated to be the most heat-sensitive stages of the reproductive growth phase in cotton [10].

# 3. Combined effects of heat and drought are enhanced in plants

Usually heat stress is coupled with limited water availability in many areas of the world. Combined effects of heat and drought stresses are not very widely discovered in cotton, although studied independently. Even though combined effects have been studied in various plants including wheat (*Triticum aestivum* L.) [11], sorghum (*Sorghum bicolor* L.) [12], grasses [13], tobacco (*Nicotiana tabacum* L.) [14], *A. thaliana* [15, 16], maize [17] and tomato [18].

It was observed that high temperatures and water limitations in combination have additive effects of individual stresses. Fundamentally, combination of both stresses aggravates the effects of individual stress. HSPs, reactive oxygen intermediate removal enzymes and many other transcripts were more actively expressed under both drought and heat stress as compared to individual stresses, when examined via transcriptome analysis [19–21]. The same mechanisms involved in response to a single stress are raised under the combined stress. The most promising result of a study conducted in Arabidopsis by Vile et al. [16] was finding a genetic variation of being greatly tolerant to the combined-stress [14].

In a study in cotton (*Gossypium barbadense* L.) by Carmo-Silva et al. [22] it was revealed that the combination of heat and drought stress adversely affect the physiological processes including growth and development compared to single stress. Cotton breeding programs need to focus on selection under both drought and heat stresses instead of focusing these stresses individually [23].

# 4. Effects of temperature stress

#### 4.1. Agronomy

Although cotton crop originated from warm-climate, the optimal temperature to accumulate biomass estimated 20–30°C [24]. Likewise, optimal window of temperature for ideal functioning of metabolism and associated enzyme should be 23.5–32°C. Exposure to high temperature (>32°C) limits the growth and development of cotton [25]. Generally, all growth stages are affected by high temperature but reproductive stage is the most sensitive and critical one. High temperature reduced the growth period and drastically impacted the agronomical aspects particularly of early maturing varieties [26]. Heat stress reduced the plant height, internodes, sympodial branches, monopodial branches, seeds per boll, boll weight, and fiber length during boll developmental process [27] depending on temperature intensity and exposure period. Suboptimal temperature significantly limited the yield formation process and decreased the boll retention. For instance, an increase of even 1°C in field than optimal-ambient temperatures, lint yield reduced by 110 kg ha<sup>-1</sup> [28]. This decline in lint yield is principally caused by a smaller boll biomass and low number of seeds produced in a boll [29] by heat-induced pollen damage and low fertility [30] and fertilization efficiency [30, 31]. Recently, Shakoor et al. [32] found that heat stress also limited the uptake of macro and micro nutrients [33].

Exposure to low average and cool night temperature (below 22°C) for extended period is also detrimental for cotton growth. Boll biomass was reported the most vulnerable yield constituent to low temperature because of late-maturity and low availability of carbohydrates induced by late planting of cotton plants [34].

#### 4.2. Physiology

Temperature stress, especially the heat stress, is considered to induce a wide number of physiological and biochemical alterations within the plant cells [3]. It has been observed that mostly the heat stress is coupled with water deficit conditions, thus by causing server injuries to plant cell membrane, disturbed protein synthesis and affecting the photosynthetic apparatus efficiency by reducing the transpiration due to stomata closure [4]. In response of this imbalanced metabolism due to induced heat stress, plants' antioxidative defense system and biosynthesis of a number of new proteins referred to as heat shock proteins (HSPs) get activated to protect plant from oxidative and membrane damage at sub-optimal temperatures [35]. Besides these prominent effects, much of other metabolic and physiological complexities such as chlorophyll synthesis, reproductive efficiency, pollination, fertilization, fiber development, carbohydrate accumulation, reduced water contents, disturbed enzymatic activities, leaf turgor pressure, water transpiration efficiency, fiber strength, fiber elongation time and fruit shedding occur in way due to a substantial increase above optimal temperatures [5, 27, 30, 31, 34, 36–46]. Being originated as hot climate crop, the ideal temperature for cotton plant growth and development lies between 20 and 32°C [35, 47-51]. Optimum performance of cotton crop in terms of maximum number of bolls and square formation, and metabolic activity is reported to occur at day and night temperatures of 30 and 22°C, respectively [49, 52]. A significant decrease in boll retention was observed by Zhao et al. [53] at high temperatures. Burke et al. [36] described the optimum temperature for pollen germination as 28°C and surges above this value is regarded as highly sensitive.

Heat stress at 40°C is reported to cause significant reductions in photosynthetic pigments, proline contents and total soluble sugars along with decreased morphological attributes in two Egyptian cotton genotypes [35]. Moreover, significant variations in number, intensity and density of SDS protein patterns were also observed for said genotypes (Giza 80 and Giza 90). Chlorophyll fluorescence is reported to be lowered at significant levels under high temperature stress or upto  $35^{\circ}$ C [54]. Photosystem II (PS-II) is regarded as the most sensitive site of the photosynthetic apparatus sensitive to heat stress, while the CO<sub>2</sub> fixation is also considered to be affected at high temperatures [55]. Rubisco activity is also reported to be affected by high temperature stress by suppressing the Rubisco activase enzyme [56–58]. High night temperatures also reduce the fiber micronaire value along with shorter fibers, whereas low night temperatures cause reductions in total cellulose synthesis and hampered boll development [59, 60]. Recently Lauxen et al. [61] observed a critical reductions in seedlings germination potential, growth and the chlorophyll contents under low (18°C) and high (35°C) temperature stress along with different levels of water availability stress.

High temperature stress is reported to affect the pollen viability and the anther indehiscence, resulting in lower seed setting rate and causing significant reductions in final yields [62]. It is

extensively documented and believed that the most viable site to be attacked during the heat spells is the photosynthetic apparatus, which is the primary site for carbohydrate production and food supply to other plant parts. The optimum value for favorable temperatures is considered as 30°C, beyond which the rise in each degree is undesirable. Schuster and Monson [63] proposed a indirect relation between the high temperature and the photosynthetic activity. Because during the stress Rubisco activity is inhibited by protecting the PS-II at high temperatures of 40°C [57]. Seedling stage is also prone to be influenced by temperatures stress in cotton plants possibly due to low germination percentage, fresh and dry shoot weights, turgor pressure, leaf soluble proteins, leaf amino acids and wax contents of epicuticle during the emergence period and early stand establishment [23, 64]. The reason for decline might be the reduced assimilated carbohydrates to newly growing tissues, which was confirmed earlier by the findings of Snider et al. [31] where a decline was observed for carbohydrate translocation to flowers from subtending leaves under stress (heat) conditions. It was also observed that heat stress tolerant cultivars exhibit the higher level of antioxidant activities prior to stress conditions as compared to susceptible cotton cultivars [30].

Declining temperatures and low light intensity due to late planting of cotton crop is attributed to the reduced yield components (boll weight and boll number), reduced fiber elongation rates, and fiber strength mainly due to lower cellulose contents and biomass accumulation [34]. Recently in Australia, Luo et al. [45], proposed a temperature modeling approach and they found that low temperatures will harm less during the early growth with delayed growing season period, whereas the impact of high temperature will be drastic to cotton crop growth, with accelerated crop development especially during the boll formation stage, which can only be catered through management options. Broughton et al. [65] observed the cotton growth and physiological response under elevated CO<sub>2</sub> and temperatures and their combinations, elevated CO<sub>2</sub> caused increase in biomass and photosynthesis, with decreased stomatal activity at ambient temperatures, however these alterations were not evident for elevated temperature. High temperature caused a significant increase in whole-plant water loss (regardless of CO<sub>2</sub> levels) thus reducing whole-plant water use efficiency Broughton et al. [65]. In a recent review by Korres et al. [66], they proposed the implications for elevated atmospheric CO2 levels by analyzing that positive effects of increased CO<sub>2</sub> on C3 crops may offset the competition for C4 weeds in C3 crops, contrastingly the C3 weeds may threaten the survival of C3 and C4 crops in tropical areas. Elevated night time temperatures cause significant increase in rate of respiration and in response there is reduced carbohydrate accumulation occurs in cotton plants [25, 67]. Pettigrew [68] evaluated six cotton genotypes for their variation in photosynthetic efficiency and heat tolerance and found a very little variation among the genotypes grown in field conditions in a very natural way of inducing heat stress with mild effects, among which only a few lines were observed with reduced (15%) photosynthetic rates. In another study conducted on 16 cotton cultivars, hypocotyl dry weight, leaf pigments and cellular respiration was found affected by heat stress at different developmental stages [69]. Ahmad et al. [70] observed a delay in reproductive stage initiation and accumulated higher thermal time in late maturing varieties as compared to shorter duration cultivars, by sowing at different thermal times, which also decreased the heat use efficiency of seed cotton yield. Alterations in plant water relations, chlorophyll pigments and antioxidant enzyme activities were reported recently under high temperature conditions (44–46°C) during square and flower initiation stages [71]. In contrast, low temperature stress (15–20°C) caused significant reductions in photosynthetic rates (37%), stomatal conductance (71%), transpiration rate (52%) and intercellular  $CO_2$  (60%), combined with flooding stress in transgenic Bt cotton [72]. Similar decrease in aforesaid physiological parameters were also observed for upland and Pima cotton, when plants employed to combined drought and heat stress, where the maximum decrease in parameters were observed at 35°C [73]. Whereas, high temperatures were also associated with high water use efficiency for both cotton species, with decreased chlorophyll *a* content and improved PS-II quantum efficiency [73]. High temperatures (~35°C) shortened the fiber rapid elongation period significantly, thus reducing final fiber length [74]. A graphical representation of high temperature impact on different growth stages of cotton is shown in **Figure 1**.

#### 4.3. Biochemistry

Effects of abnormal temperature on cotton crop are more pronounced during the reproductive stages namely the boll formation and fiber development. Fiber length, uniformity, strength and the micronaire values are affected by high daytime temperatures, thus affecting the fiber quality [75]. Whereas, the optimal temperature (night) for fiber elongation was proposed

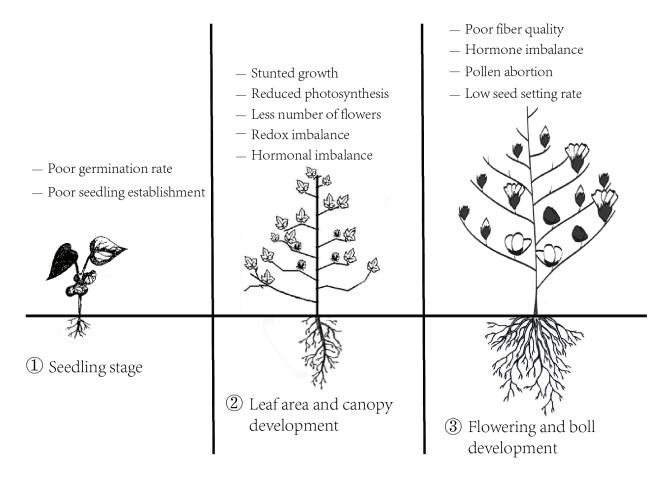


Figure 1. Effect of high temperature on agronomic and physiological attributes of cotton at various developmental stages.

between the range of 15 and 21°C [76]. Major osmotically active solutes in the cotton fiber includes soluble sugars, malate and potassium ( $K^+$ ), contributing the 80% of fiber sap [77–79], and these components are extensively reported to be influenced by suboptimal temperatures. Moreover, carbohydrate assimilation during boll development in cotton plant is primarily (> 60%) comes from the subtending leaf of boll [80], and this leaf also influenced badly during the hot spells of temperature and drought stress, thus affecting the photosynthetic rate which ultimately imbalances the carbohydrate production in leaf [31]. Recently Chen et al. [81] observed that high temperatures combined with waterlogging conditions inhibits the cell elongation due to influenced osmolyte composition in a newly developing fiber of cotton crop. Further they also confirmed that reduced fiber elongation occurred by alterations in the osmotically active solutes, sucrose, malate and K+ present in fiber sap, which mainly due to waterlogging conditions [81]. Whereas, the high temperatures (34.1/29.0°C) accelerated the early fiber development with reduced fiber elongation periods, mainly due to the altered fiber sucrose content by expression of sucrose transporter gene GhSUT-1 [81]. Similarly, several genes are reported to induce the anther indehiscence, among which only a few (5 genes) are able to control the carbohydrate metabolism and programmed cell death [82].

Temperature stress may cause deleterious effects at cell and molecular level, their networking and also during protein synthesis [46, 83]. Due to which a number of cellular abnormalities, metabolic imbalances, instable homeostasis and complex molecular reprogramming can be observed at transcriptional and post-transcriptional levels [62]. As we described earlier that heat stress is normally taken together with water deficit (drought) conditions normally in cotton plants thus fortifying the stress impact from both abiotic sources. Sarwar et al. [42] recently confirmed the accumulation of HSPs in response to drought conditions in transgenic cotton containing the HSP gene (GHSP26), as compared to wild type. They also observed increased level of leaf water contents (69%), and physiological attributes (photosynthesis, stomatal conductance, transpiration rates and osmotic potential) in the transgenic cotton plants [42]. Cotton genotypes under heat stress, during their evaluation for stress tolerance, are reported to induce the expression of certain HSPs in tolerant genotypes as compared to the susceptible ones [35, 64, 84]. Recently, Wang et al. [85] characterized a cotton abiotic stress inducible TPS gene GhTPS11, the over expression of which increased the sensitivity of transgenic Arabidopsis seeds under low temperature stress which resulted in increased level of T6P or trehalose. Tolerance of 58 cotton genotypes were assessed for heat stress recently in Pakistan based on some agronomic and physiological parameters, and it was observed that genotypes showed variations to heat tolerance on the basis of affected relative cell injury percentage and heat susceptibility index [86], thus confirming the plausible damage to cell membrane due to stress. Cell membrane thermo-stability (CMT) was proposed by Sullivan [80] as distinct criteria for heat stress assessment. CMT was significantly reduced under high temperature stress ranging from 44 to 49°C as compared to normal field temperatures (37-39°C) in a Pakistani cultivar MNH-886 during 2013-2014 [87]. Iqbal et al. [26] recently evaluated some genes responsible for drought (four) and heat stress [76] in field grown cotton for MAS, they did not found any variations for studied genes responsible for heat stress among the genotypes, thus recommended to include both traits (heat and drought) for selection. Chlorophyll contents and PS-II potential photochemical conversion efficiency of top fourth leaf decreased with increasing

ground water-table and high temperature, along with significant alterations in SOD, POD, CAT and MDA activities due to heat stress at flowering and boll formation stages in cotton [88]. Song et al. [89] have identified sensitive stages of square development at high temperature upto 40°C, they observed the inhibition of pollen tube growth was more pronounced at temperatures above than 35°C, which adversely affected the cotton yield due to heat stress at square development stage. They confirmed that stages from sporogenous cell to tetrad stage (square length < 6.0 mm) was much susceptible to heat stress Song et al. [89]. Snider et al. [90] described that ability to tolerate heat stress could be influenced by plant developmental stages, irrespective of any heat or drought stress, as they characterized this phenomenon for Gossypium hirsutum by evaluating PS-II quantum yield, its efficiency and quantum yield of electron transport. Wang et al. [91] suggested that brief water logging conditions with elevated temperatures can improve sucrose composition and its accumulation in subtending leaf, mainly by improved photosynthesis and inhibition of sucrose degradation. The defensive system of a moderately tolerant cotton cultivar from Pakistan could not protect cellular membrane of stressed plants under extreme temperatures (38 and 45°C) [41]. Recently in Pakistan, Khan and his coworkers [92] have screened out some cotton cultivars/lines for heat stress tolerance, and they found significant variation among the genotypes for the evaluating criteria of relative cell injury percentage. Increased night temperatures (30°C) were reported to increase the pistil glucose, sucrose and starch concentrations, whereas the leaf starch concentrations were reduced, which [91] was seen protected by efficient leaf antioxidant metabolism [93]. Besides the deleterious effects of high temperature stress on physiological and biochemical aspects, the chilling temperatures (below 20/15°C) also causes significant alterations and oxidative damages to cotton plant cellular and molecular mechanisms, the extensive review for which (chilling stress) is recently published by Holaday et al. [94], in which the authors described in detail the prominent effects on cotton photosynthetic apparatus and its networking complex metabolism pathways. A pictorial representation of mechanisms of temperature stress tolerance or susceptibility is shown in Figure 2.

#### 4.4. Quality

Suboptimal temperature occurrence for few days may affect the cotton yield quality during any time of the growing season. Under stress like excessive heat or moisture, low temperature or nutrients than optimal requirements, cotton shed some squares, flowers, and bolls to ensure survival under unfavorable conditions which caused a significant decline in fiber quality [95]. Cotton yield and fiber quality related aspects (fiber strength, elongation, fineness, and micronaire value) negatively impacted under higher temperature [29]. Although, all stages of fiber formation are affected by temperature extremes, Initial fiber elongation period is most vulnerable to temperature stress. Fiber properties are dependent on photosynthates present in fiber cell walls which are vulnerable to fluctuations in temperature [68]. Suboptimal temperature generally impedes the cellulose synthesis process, and therefore fiber elongation and maturity, consequently, fiber of poor quality is produced [25]. Optimal temperature for fiber uniformity and micronaire was recognized 26°C, and reduced at higher temperature. Moreover, Optimal temperature for fiber length was recognized 18–22°C, and reduced at higher temperatures [96]. Fiber quality is also constrained by low temperature in several cotton-growing regions [97]. Temperature Extremes in Cotton Production and Mitigation Strategies 73 http://dx.doi.org/10.5772/intechopen.74648

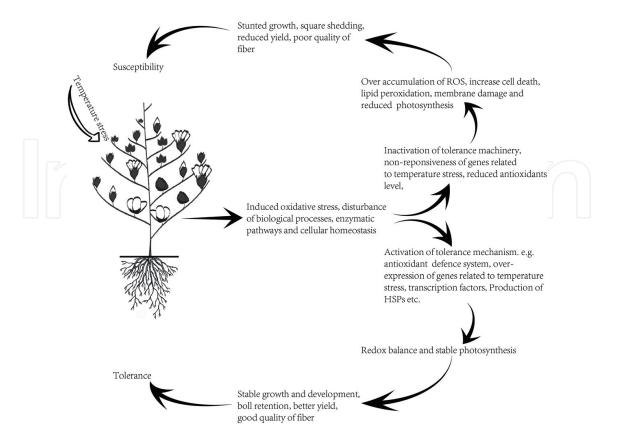


Figure 2. Physiological and biochemical mechanisms of tolerance or susceptibility under temperature stress in cotton.

Exposure to average daily temperature (20.6°C) at fiber elongation stage significantly reduced the fiber quality by changing the expression of proteins involved in cell wall loosening and biosynthesis, osmotic adjustment, and cytoskeleton homeostasis [98].

# 5. Mitigation strategies to avoid harmful effects of temperature stress

#### 5.1. Agronomic practices

To adopt temperature stress, strategies should be applied according to site-specific conditions. Like, growing the verities of thick cuticle and waxy surfaces that can reflect solar radiation to reduce the impact of heat stress [99]. However, most of the verities are good absorber of solar-radiations, which can increase the stress impact. Recently, it was reported that night temperatures would increase further in future [100] that could adversely affect the cotton productivity. Higher temperatures also limited the cotton growth and development by inducing directs impacts of heat stress, and indirectly by exposing plant to drought conditions. By altering row-spacing under rain-fed systems can increase availability of soil water for plants, impact the lint yield, increase fiber quality, and reduce the level of unpredictability associated with production under stress [101]. Irrigation scheduling based on plant-needs accessed with canopy temperature sensors can also play a crucial role in ameliorating the negative impact of temperature and drought stress [102].

Planting time adjustment is most crucial strategy to addressing temperature stress. Recent finding suggested that changes in planting time significantly affect the cotton growth, lint yield, efficacy of nitrogen utilization and assimilate supply to reproductive organs [92]. Altering planting time would have minor impact on cotton yield irrigated farming systems, but substantial influences on cotton yield of rain-fed farming systems [103]. Adjustment of planting time therefore can ameliorate the negative impacts of stress by adjusting it according to specific growing regions.

Exogenous application of natural and synthetic plant growth regulators [104] is an important and quick agronomic approach to reduce the negative impact of temperature stress [104]. PGRs (Hydrogen peroxide, ascorbic acid, salicylic acid, Moringa leaf extract) significantly enhanced the cotton yield under heat stress by potentiating the cell membranes and enhancing the antioxidant defense [41]. Likewise, exogenous application of benzoic acid improved the cotton performance exposed to heat stress by enhancing the growth rate and nutrients uptake [32].

#### 5.2. Genomic approaches

#### 5.2.1. Marker-assisted selection and identification of QTLs for crop improvement

Molecular marker-assisted selection (MAS) is preferred over visual selection because it is time and cost effective. MAS is a powerful strategy to accelerate the crop breeding for tolerance against biotic and abiotic stresses [105, 106]. Study and development of molecular markers that are linked to the chosen traits [107, 108] and utilization of indirect selection of required loci using molecular markers is a proficient selection tool. Numerous markers have been developed in the recent past like restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs), and simple sequence repeats (SSRs) to be utilized in breeding programs via MAS [106].

Usually molecular markers are not developed from the desired genes. On the other hand, development of functional markers (FMs) is generally based on observed polymorphism in transcribed regions of the functional target genes, which make these markers suitable to develop a complete correlation with gene function. Functional markers enable precise selection of target genes [109–111]. However, utility of molecular markers for subsidiary selection is restrained for improvement of traits with marker-assisted backcrossing (MABC) of key genes [112]. Abiotic stress tolerance being the quantitatively inherited traits, which implicates introgression of many genes, is logically not feasible for MAS in breeding programs [113, 114]. In Addition, the requisite of mapping important marker–trait relations through breeding pools, in contrasting environments first, or selection for numerous cycles is another disadvantage of MAS approaches. Marker-assisted recurrent selection [101] approach is a recent strategy comprising of several cycles of the subsidiary selection, is promising to achieve the required occurrence of alleles of target quantitative trait locus (QTL) [115].

Moreover, another modern approach is genome-wide selection (GS) which utilizes the collective influence of genome-wide markers on a trait, which leads to pyramiding promising alleles for minor-effect QTLs [115–117]. Previous knowledge about QTL regulating the required trait is not required, which is the foremost benefit of GS. Single nucleotide polymorphisms [110], new-generation markers, are abundant, robust and cost effective, are preferred over the conventional molecular markers [118]. Furthermore, these markers can be automated and can competently screen huge populations [119]. Research to identify SNPs for establishment of functional SNPs for prior selection, and for development of high-resolution SNP chips using deep sequencing for association genetics studies is going very fast [118, 120, 121]. High-throughput genotyping of markers and the accessibility of economical, next-generation sequencing platform can effectively facilitate genome-wide selection for crop enhancement in the near future [116, 122, 123]. Recently Fu et al. [124] made an endeavor to develop a substitute for conventional genomic selection using function-associated specific trait FAST SNP markers that can be utilized to accomplish trait-specific prediction more precisely. Continual work to establish better options will lead to improved marker-based evaluations for quantitative traits in molecular plant breeding.

Complex genome of allotetraploid cotton (*G. hirsutum* L.) and its narrow genetic base needs exhaustive work to obtain necessary polymorphism for marker based breeding. In cotton, high throughput markers can be developed utilizing the sequenced cotton genomes coupled with next generation sequencing (NGS) technologies. The perceptions of MAS, QTL mapping and genetic diversity have been coined into genomic selection, linkage disequilibrium and association mapping respectively [125].

#### 5.2.2. Examples of developing markers linked to temperature extremes for MAS in cotton

Mohamed and Abdel-Hamid [35] observed the influence of heat stress at morphological, biochemical and molecular levels in four cotton (*Gossypium hirsutum* L.) genotypes when grown at 30°C for control plants and at 40°C for heat stress treatment. Plants under stress treatment shown a significant impact of heat stress on morphological traits, on the number and intensity of protein bands and activity of isozymes as compared to control plants. This data coupled with RAPD analysis shown two genotypes (Giza 85 and Giza 92) as tolerant genotypes which can be introduced in breeding programs [35].

CAPS and dCAPS the SNP markers developed from specific genes are helpful in molecular breeding of crops. *G. hirsutum* and *G. barbadense* (cultivated allotetraploid cotton species) have discrete fiber quality and many agronomic traits. Kushanov et al. [126] performed the examination and characterization of GSTs of the HY5, PHYA1and PHYB genes of *G. hirsutum* and *G. barbadense* by comparative analysis. They developed one HY5-specific Hinf I dCAPS, one PHYA1-specific Mbo I/Dpn II CAPS and one PHYB-specific Alu I dCAPS cotton markers. These markers could distinguish the two allotetraploid genomes (AD1 and AD2) successfully when tested in parental genotypes of 'Pima 3–79', 'Texas Marker-1' ('TM-1') and their F1 hybrids. PHYA1 gene was mapped on chromosome 11 of A-sub-genome, PHYB gene on chromosome 10 of A-sub-genome, and HY5 gene on chromosome 24 of D-sub-genome, on the reference 'TM-1' x'Pima 3–79' RIL genetic map. The genetic linkage map region containing HY5 and phytochrome-specific markers were found linked with key fiber quality and flowering time traits. In previous studies Kim et al. [127] found the Phytochrome B as a key photoreceptor governing the initiation of cold-stress signaling in light response. These gene markers are valuable candidates in marker-assisted selection (MAS) programs to promptly

introgress *G. barbadense* phytochromes and/or HY5 gene (s) into *G. hirsutum* cotton genotypes or vice versa [126].

#### 5.3. QTL mapping for heat tolerance in cotton

#### 5.3.1. QTLs for heat and drought tolerance

Deriving a connection between a genotype and phenotype is very challenging in the environmental context. Scrutinizing the variations in compound traits either by identifying QTLs in a population developed by crossing two parents or through a genome-wide association study (GWAS) conducted on a set of diverse and distinct individuals, is mainly aimed for identification of alleles responsible for variation in a concerned phenotype. Therefore, studying QTL is vital for recognition of desired genomic regions that can be utilized in molecular breeding programs for improving cotton genetically.

In a study conducted by Ulloa et al. [128], two QTLs were identified for stomatal conductance under high temperatures and irrigated field conditions. Enhanced stomatal conductance provides a cooling effect and in that way, a sort of heat escaping mechanism thus mitigating losses in yield. These findings can be helpful in investigating genetic elements to enhance cotton productivity in warm and dry environments. Studies for the identification of QTLs related with a combination of abiotic stresses are very meager; however, field-based studies relevant to attaining tolerance in field conditions must be emphasized [23].

Certainly, cotton is grown under both elevated temperatures and water shortage. It is also happening because of climate changes globally. Development of varieties, which are tolerant to drought and heat stresses in combination, should be considered by breeders. In a study by Dabbert [129] 138 QTLs for two agronomic and six fiber traits were identified in three separate experiments. Heat sensitive parents were found to have high number of beneficial alleles controlling lint yield and seed cotton yield rather than the heat-tolerant parents. Nonetheless, for polygenic traits a less number of QTLs can be identified in small mapping populations. For the development of tolerant varieties against combined drought and heat stress genomic selection is more practicable in cotton [129].

# 5.3.2. QTLs for freezing tolerance

Although QTLs linked to low temperature tolerance have been reported in many plants like tomato [130], Rice [131], wheat [33]; However in cotton studies related to identification of QTLs for freezing tolerance are scanty.

#### 5.4. Identification of genes responsive to temperature extremes

#### 5.4.1. Genes for heat tolerance

Possibly, identification of genes for improved yield is the best choice for yield enhancement under optimum production conditions. Under stress conditions those plant perform better which were growing well under high inputs environment [132]. Many studies have been conducted to identify genes involved in tolerance to temperature extremes (**Table 1**) [88].

Identified gene/transcripts	Involvement in abiotic stress tolerance	Species used		Reference
GhDREB1	Cold stress response (transformed into tobacco)	(G. hirsutum)		Shan et al. [138]
2 Phospholipase Da (PLDa) genes	Responsive to cold stress	(G. hirsutum)		Kargiotidou et al. [137]
GhTIP1	Cold tolerance	(G. hirsutum)		Li et al. [136]
GhAGP31	Cold tolerance	(G. hirsutum)		Gong et al. [139]
25 ESTs	FPGS3, GhHS126 and GhHS128, responsive to high temperature	Heat susceptible (Nazilli 84S) and tolerant (Stoneville 453, BA 119) cultivars ( <i>G. hirsutum</i> )		Demirel et al. [133]
94 Heat Shock Protein 20 encoding genes	16 GhHsp20 genes induced with heat stress, and eight genes upregulated by combined abiotic stresses and phytohormone usages	(G. hirsutum)		Ma et al. [132]
miRNA encoding genes	319 known miRNAs and 800 unique miRNAs were recognized, and 168 miRNAs were expressed differentially among different temperature treatments	(G. hirsutum)		Wang et al. [85]
Heat stress transcription Factors HSFA2, HSFA1b Heat shock proteins GHSP26, HSP101, HSC70–1 encoding genes	Heat stress	Heat-sensitive (ST213 and ST4288) and heat-tolerant (VH260 and MNH456) genotypes of cotton in <i>G. hirsutum</i>		Zhang et al. [134]
HY5, PHYA1 and PHYB genes (CAPS and dCAPS markers development from GSTs of the genes)	Cold-stress signaling in response to light	<i>G. hirsutum</i> and <i>G. barbadense</i>		Kushanov et al. [126]

Table 1. Genes identified in cotton involved in tolerance to temperature extremes.

In cotton cultivars, dissecting the genetic pathways of heat stress responses can help in establishing heat tolerance. Demirel et al. [133] made an effort to determine genes, which were showing response to heat stress in cotton. They used susceptible (Nazilli 84S) and tolerant (Stoneville 453, BA 119) cultivars and sequences of 25 expressed sequence tags (ESTs) were considered for gene homology. Remarkable homology with known genes was found for 16 ESTs, while 8 ESTs were similar to cDNA clones which were not annotated and 1 EST was not showing similarity to any well-known gene. IAA-ala hydrolase (IAR3) and quantitative real-time PCR analysis of the genes revealed that folylpolyglutamate synthase (FPGS3), and two ESTs (GhHS126 and GhHS128) which were not annotated were constantly up-regulated under short- and long-term both heat stresses. The ESTs can be further utilized in developing and enhancing heat tolerance in cotton and other plants. Furthermore, GhHS126 and GhHS128 ESTs can be part of the new favorable genes for heat tolerance [133].

Heat Shock Protein 20 [132] is important for growth and development under abiotic stresses in higher plants. Ma et al. [132] identified 94 GhHsp20 genes in *G. hirsutum*, and clustered them

in 14 subfamilies phylogenetically. Eighty-two GhHsp20 genes were being expressed in at least one examined tissues, which revealed that the GhHsp20 genes contribute in physiological processes and growth in cotton. Two third of the genes were found involved in heat stress response whereas compound stresses induced other 15 genes. The qRT-PCR analysis inveterate the induction of 16 GhHsp20 genes with heat stress, and upregulation of eight genes by combined abiotic stresses and phytohormone usages was confirmed [132].

The endogenous miRNAs, which are a type of sRNAs are involved in transcriptional and posttranscriptional regulation in plants during development and adjective responses to stresses. In response to abiotic stresses including drought, salt, heat, cold, and oxidative stresses, mi RNAs are found to be under or over expressed. MicroRNAs (miRNAs) are a type of non-coding, endogenous RNAs, which control the specific gene's expression by degradation of RNA or limiting the translation. Wang et al. [85] used small RNA and mRNA degradome sequencing to recognize mi RNAs which are high- and low-temperature stress-responsive and targets genes for them in cotton (*G. hirsutum*). Totally, 319 documented miRNAs and 800 unique miRNAs were recognized, and 168 miRNAs were expressed differentially among different temperature treatments. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes revealed that commonly the miRNAs were from genes, which contribute in oxidation–reduction reaction, response to hormone stimulus, plant–pathogen interaction, photosynthesis, and plant hormone signal transduction pathways [85].

Utilization of molecular tools and genetic engineering in breeding for heat tolerance can minimize the complications of polygenic nature of the traits. Zhang et al. [134] conducted a comparison of expression of certain heat-stress responsive genes between heat-sensitive (ST213 and ST4288) and heat-tolerant (VH260 and MNH456) genotypes of cotton in *G. hirsutum*. Orthologs of particular Arabidopsis genes involved in heat-stress response including three heat shock proteins, two heat-stress transcription factors, and the general stress response genes: calcium dependent stress responder, ANNAT8 and ascorbate peroxidase were studied in cotton. Real time qPCR analysis after heat stress treatment revealed that all genes, excluding the heat-shock protein GHSP26, were entirely induced in the heat-tolerant lines of the genotype VH260 as compared to MNH456. Resilient tolerance to heat stress in VH260 can be attributed to prompt sensing of heat stress and timely induction of several mechanisms functioning in coordination to secure the plants against oxidative stress, protein denaturation and membrane damage leading towards decreasing yield losses and improved boll maintenance during heat stress [134].

#### 5.4.2. Genes for cold tolerance

Plants show various responses to encountered environmental stresses. Exposure to low temperature causes expression of numerous genes coding for the proteins that enhance low temperature tolerance via ABA-dependent and ABA-independent pathways [135]. C/DRE, which is a cis acting element shows response to low temperature separately from action of ABA [127].

Study of Phytochromes and aspects involved in their signal transduction are important due to their involvement in plant development and in numerous genetic/biochemical pathways like in plant flowering and architecture, cotton fiber quality, yield potential and productivity, regulation of nitrate reductase, in fungal disease resistance, salt tolerance, in cold/freezing and drought tolerance [126]. Kim et al. [127] investigated the involvement of phytochromes in facilitating light signaling associated with cold treatment as a photoreceptor for activation of gene expression in response to cold through C/DRE in *A. thaliana*. They found phytochrome B as key photoreceptor controlling the initiation of cold-stress signaling in light response.

Aquaporins are a class of proteins which were reported to play critical roles in plant abiotic stress tolerance. In cotton, a tonoplast intrinsic protein [6], GhTIP1, was reported to enhance the cold tolerance under freezing conditions [136].

Kargiotidou et al. [137] identified and characterized two Phospholipase Da (PLDa) genes from cultivated tetraploid cotton (*G. hirsutum*). Three exons and two introns were observed in genes. A 98.6% homology was observed in both GrPLDa and GaPLDa with their ORFs encoding a polypeptide of 807 amino acids with an expected molecular mass of 91.6 kDa showing an 81–82% homology with PLDa1 and PLDa2 of *A. thaliana*. At the 5′ end a potential alternative splicing incidence was noticed that did not produce alternative ORFs yet. Genes were induced at cold stress (10°C or less) treatment which was declined to control conditions (growth temperature 25 or 22°C) if plants were adapted at 17°C prior to applying cold treatment. Isoforms were differentially expressed when acclimatized to cold and when under cold stress, light was involved in regulation in expression which was attributed to the products of lipid hydrolysis by the endogenous PLDa changing lipid species and a deviation in levels of the signaling molecule phosphatidic acid (PA) after acclimation or cold stress [137].

The transcription factors C-repeat binding factors/dehydration-responsive element binding proteins (CBFs/DREBs) are involved in controlling the expression of many stress-inducible genes. After screening the cDNA library a cDNA clone, named GhDREB1, was identified from cotton (G. hirsutum). Results of northern blot analysis revealed that low temperature and salt stress were causing enhanced synthesis of mRNA of GhDREB1 while effect of abscisic acid (ABA) or drought stress was insignificant in cotton seedlings. Over expression of GhDREB1 in transgenic tobacco (Nicotiana tabacum) plants exhibited improved tolerance to low temperature than wild-type plants with enhanced leaf chlorophyll, net photosynthetic rate and proline concentrations. Conversely, the transgenic tobacco plants showed minimum growth and late flowering under normal growth conditions. Interestingly, the transcripts of GhDREB1in seedlings of cotton down regulated by treatment of gibberellic acid (GA3). Promoter analysis of the GhDREB1 gene showed that one low-temperature and four gibberellin-responsive elements were present in promoter. Green fluorescent protein (GFP) signal intensity or β-glucuronidase (GUS) activity caused by the GhDREB1 promoter was remarkably enhanced by low temperature but inhibited by GA 3. These findings proved that GhDREB1 works as a transcription factor and is involved in enhancing cold tolerance also affecting growth and development of plant via GA3 [138]. Another gene from Gossypium hirsutum, GhAGP31, expressing mainly in roots was shown to play important role in tolerance to cold stress during early seedling development [139].

#### 5.5. Utilization of wild species in breeding programs to enhance traits

Homogeneity at genomic level in cotton germplasm is one of the main cause of halted or dropped cotton production around the world rendering cotton crop prone to biotic and abiotic

stresses. Certain wild species possess unique traits including resistance to drought and heat. Valuable traits can be introduced in cultivated cotton varieties using hybridization of various species like, *G. arboreum*, *G. herbaceum*, *G. gossypioides*, and *G. laxum* with *G. hirsutum* and/or *G. barbadense*, afterwards using culture media to raise embryos which may ease in breaking cytogenetic hurdles. This method can be utilized to broaden the genetic base and also for transferring genes involved in traits that are absent in the cultivated species [70]. Enormous variation is present among the cotton germplasm for cold tolerance. Bolek [140] screened 106 cotton genotypes including *G. hirsutum*, *G. barbadense* and *G. herbaceum* for cold tolerance regarding germination efficiency, and found that *G. barbadense* had higher cold tolerance than other species. Thus these genotypes can be utilized in distant breeding program to enhance cold tolerance of our cultivated cotton cultivars.

Cotton genome sequencing accompanied with novel experimentations like nested association mapping based studies, and utility of TILLING populations can be more prolific for cotton breeding. Information generated in genomes, which are well studied, can be interpreted in less explored genomes with the help of comparative mapping. Improved knowledge about evolutionary relations of cotton and Arabidopsis have facilitated deciphering respective gene localization in both genomes which can lead to isolation of full length genes in cotton after getting knowledge about their function from Arabidopsis. This information will also facilitate improvement of translational genomic tools after sequencing of cotton genomes and also in elaborating biological pathways. The sequenced cotton genomes G. arboreum [141], G. raimondii [149], G. hirsutum [142] and G. barbadense [143] can be explored for trivial variations at nucleotide level that could be involved in controlling specific traits of cotton. The problem of narrow genetic base of cotton, which is the result of rigorous selection for desired traits, can be solved by getting alleles from wild ancestors [144]. Tetraploid cotton of exotic areas are comparatively heat and drought tolerant. For example, the arid, rocky and clay coastal plains of Hawaii are homeland of GT (http://kalama.doe.hawaii.edu/hern95/pt009/Ann/mccnativeplants.html). Interspecific crossing between GT and GH could produce limited water stress tolerant progenies [145]. A significant variation in WUE, dry matter accumulation, root length and heat tolerance was found amid exotic GH lines [146]. Although development of interspecific hybrids and their utility in breeding programs is very challenging [147].

# 5.6. Utilization of modern techniques to improve cotton genome against high or low temperature stresses

Whole genome sequencing has revolutionized the genome science. Genotyping-by-sequencing (GBS) is also an alternative lower cost method to identify and score multitude of genome-wide single nucleotide polymorphism (SNP) markers through multiple individuals from miscellaneous populations. Moreover, remote sensing and proximal sensing technologies are promising for the speedy, non-invasive measurement of canopy traits related to the response of cotton to drought and heat stresses in the field. Satellite and aircraft based systems are very informative in context to spectral reflectance and canopy thermal emittance data to be utilized in observing the growth patterns and physiological responses of cotton cultivars grown in field conditions. Hand-held, noncontact sensors when passing through field plots on foot can perform proximal sensing in cotton [23]. Expansion of breeding programmes at genetic level

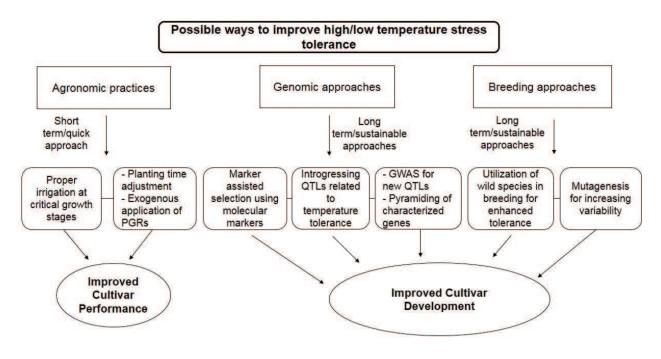


Figure 3. Possible ways to improve high and/or low temperature stress tolerance in cotton.

is significant. Utility of conventional QTL mapping as well as genome-wide association mapping is required to enhance tolerance to temperature extremes. In addition, global expression profiling techniques together with RNA-Seq and miscellaneous omics platforms can be helpful in understanding the fundamental mechanism and selection of the candidate gene (s) for downstream utility. These new techniques are immensely helpful in plant breeding [148]. A schematic diagram for various short and long term possibilities to improve high and low temperature stress tolerance in cotton are shown in **Figure 3**.

# 6. Conclusion

Changing climate has been creating extreme temperature in many countries around the globe. Temperature stress, more importantly, high temperature has multiple effects on cotton growth and production affecting its yield negatively. In this chapter, we concluded that adopting multiple strategies rather than relying on a single approach is imperative to minimize the losses to cotton production. Breeding temperature tolerant cultivars would be a sustainable and cheapest approach to get good produce under extreme temperature situation. For this, selection of good parents, wild relatives and identification of target genes or markers are of prime importance to start a breeding program.

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# **Conflict of interest**

We confirm that there are no conflicts of interest.

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