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# MOLECULAR BIOLOGY AND PHYSIOLOGY

## Heat-tolerance in Cotton Is Correlated with Induced Overexpression of Heat-Shock Factors, Heat-Shock Proteins, and General Stress Response Genes

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### ABSTRACT

**Breeding for heat tolerance is complicated by the polygenic nature of the trait and genetic engineering awaits delineation of the molecular mechanisms. To understand heat-tolerance mechanisms in cotton (*Gossypum hirsutum* L.), the world's most important natural fiber crop, this study compared expression of selected heat-stress response genes between heat-tolerant (VH260 and MNH456) and heat-sensitive (ST213 and ST4288) cotton genotypes. Cotton orthologs of selected *Arabidopsis* heat-stress response genes included two heat-stress transcription factors, three heat-shock proteins, and the general stress response genes: ascorbate peroxidase and the calcium-dependent stress responder, ANNAT8. The seedlings and flowering plants of each genotype were exposed to heat (38°C) for 1 to 3 hours or days, respectively, followed by RNA isolation from the seedlings and ovaries of flowering plants for gene expression analysis by real-time quantitative polymerase chain reactions. All genes, except the heat-shock protein GHSP26, were found to be induced exclusively in the heat-tolerant lines. The genotype VH260, which reportedly is tolerant to extreme heat (45°C), was found to display much higher induction of these genes than MNH456, the heat-tolerance level of which has not been thoroughly studied. Further, VH260 showed high gene induction in both seedlings and ovaries, whereas MNH456 showed lower induction levels. Strong heat tolerance in VH260 most likely is based on early induction of multiple mechanisms coordinately functioning to defend the plants against membrane damage, protein denaturation, and oxidative stress, in addition to rapid sensing of heat stress to signal the defense processes at the flowering stage towards minimizing yield losses and increasing boll retention during heat stress.**

The increase in greenhouse gas emission is associated with the increase in the earth's surface temperature, which adversely affects agriculture and crop productivity through heat and drought stress (IPCC, 2014). Heat stress, as with other abiotic stresses, causes numerous biochemical and physiological changes impacting photosynthesis and overall productivity of plants. Stress tolerance is a polygenic complex trait that is often found in noncultivated genotypes. However, breeding stress tolerance in cultivars is inherently unpredictable and time consuming (Dabbert and Gore, 2014) and often confounded by negative epistatic plant phenotype.

Plant response to heat stress has been studied in both model plants and crop plants. It is accepted generally that the increase in reactive oxygen species during heat stress causes both cellular damage and downstream signaling leading to heat tolerance (Pucciariello et al., 2012; Qu et al., 2013). The heat-tolerance mechanisms in plants have been understood only partially. However, the central role of heat-shock proteins (HSPs) and heat-stress factors (HSFs), in addition to the role of antioxidant enzymes to protect against oxidative damage, is well established (Kotak et al., 2007). Additional mechanisms potentially contributing to heat tolerance involve phytohormones, second messenger molecules such as Ca++, and a variety of transcription factors (Hasanuzzaman et al., 2013; Kotak et al., 2007). Among the downstream processes, protection against oxidative damage and protein aggregation during heat stress are crucial for maintaining cellular membrane integrity and photosynthesis (Allakhverdiev et al., 2008). Therefore, overexpression of HSPs initially appeared to be a promising approach for engineering acquired heat tolerance in plants; however, only limited success has been reported in past

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decades (Chauhan et al., 2012; Montero-Barrientos et al., 2010; Wang et al., 2016), and no field-tested, heat-tolerant, transgenic line has been reported to date. These observations suggest that a single heat-tolerance mechanism might not be sufficient and additional mechanisms will be needed to generate durable heat-tolerant cultivars.

Cotton grows in warm temperatures (20 to 30°C); however, at 35/25°C day/night temperature, photosynthesis and flower survival is affected severely (Reddy et al., 1991). Most ecosystems where cotton is grown experience moderate to extremely high day and night temperatures, which are considered the primary factors behind yield losses (Oosterhuis and Snider, 2011; Reddy et al., 1991). Therefore, heat-tolerant varieties of cotton are important for sustaining cotton production. To understand induced heat-tolerance mechanisms in *Gossypium hirsutum* L., this study compared the expression of selected heat-stress response genes in the seedlings and flowering plants of two heat-tolerant and two heat-sensitive *G. hirsutum* genotypes upon heat exposure (38°C). These genes are reportedly associated with three distinct heat-tolerance mechanisms: prevention of oxidative damage (antioxidant protection), prevention of protein aggregation, and heat-sensing/defense signaling (Kotak et al., 2007). This study found that rapid induction of all three mechanisms is associated with strong heat tolerance in the cotton variety VH260, which would serve as a good source of heat-tolerance alleles for genetic engineering of cotton.

## MATERIALS AND METHODS

**Plant Materials and Temperature Treatment.** Two experiments were performed to evaluate the effect of heat stress on seedlings and flowering plants of cotton (*G. hirsutum*). Four genotypes were used: two heat-tolerant cultivars from Pakistan, VH260 and MNH456, and two heat-susceptible cultivars, ST213 and ST4288B2F (Barfield, 2014). Seeds were planted in 2-L pots containing Sunshine Mix 1 (Sun Gro Horticulture Inc., Bellevue, WA), and placed in two growth chambers (Conviron E15 chamber in Rosen Center, and Conviron PGR15 chamber in Altheimer Center, University of Arkansas, Fayetteville, AR). Day/night temperatures of 32/24°C and photoperiod of 14/10 h (day/night) at photosynthetic photon flux density of 500 μmol $m^{-2}s^{-1}$ were applied. Plants were irrigated with deionized water until 4 d after germination, followed by daily application of quarter-strength Hoagland's solution. Two weeks after planting, half of the seedlings were randomly transferred to another growth chamber with day/night temperature of 38/24°C for the heat treatment. For the flowering plants, at approximately flowering time (6 wk after germination), half of the plants were randomly transferred to another growth chamber with day/night temperature of 38/24°C. For the physiology experiments, 1 wk of heat treatment was applied on seedlings or flowering plants before taking measurements. For the gene expression experiments, 1 to 3 h of heat treatment on seedlings and 1 to 3 d of heat treatment on flowering plants was applied before collecting tissue for RNA isolation.

**Selection of Heat Tolerance Genes.** Literature related to plant heat-tolerance genes was searched and reviewed. National Center for Biotechnology Information (NCBI) database and The *Arabidopsis* Information Resource (TAIR) mainly were used as the literature sources. Genes, mainly *Arabidopsis* and cotton genes that have been described to enhance heat and drought tolerance were selected and listed for further identification. The selected genes were checked through *Arabidopsis* e-FP Browser (e-FP: http://www.bar.utoronto.ca/) for the gene expression pattern, including expression time, organ, and quantitative levels. Highly expressed genes in seedlings and open flowers were selected for further examination.

**Identification of Cotton Orthologs.** NCBI and Cotton Genome Database (Cotton DB) were used to find cotton orthologs of *Arabidopsis* heat-tolerance genes using Basic Local Alignment Search Tool (BLAST) (Table 1). After finding the protein sequences of heat-tolerance genes, corresponding DNA sequences were aligned with cotton Express Sequence Tag (EST). E-value and identity (%) were the main parameters that were considered in selecting heat-tolerance genes. The genes that had E-values lower than E-70 and identity higher than 60% were considered to be good candidates as cotton orthologs. The cotton sequence coverage of selected genes also was considered as a supplemental parameter. After alignment, the related cotton ESTs sequence were used to make alignment with corresponding gene nucleotide sequences to find the highly conserved regions. Sequences ≥ 500 bp were selected for primer design by Primer 3 and Net Primer (frodo.wi.mit.edu/primer3/ and www.premierbiosoft.com/netprimer/index.html).

**Table 1. Genes selected for real-time PCR analysis**

| Type | Genes | Gene ID | Source | Function | Identity | Reference |
|---|---|---|---|---|---|---|
| Heat stress transcription factors | HSFA2 | At2g26150 | *Arabidopsis thaliana* | Heat/drought tolerance | 57% | Nishizawa et al. (2006) |
| | HSFA1b | At5g16820 | *Arabidopsis thaliana* | Heat response | 81% | Yoshida et al. (2011) |
| Heat shock proteins | GHSP26 | DN779846 | *Gossypium arboreum* | Heat/drought tolerance | 82% | Maqbool et al. (2010) |
| | HSP101 | At1g74310 | *Arabidopsis thaliana* | Thermo-tolerance | 90% | Hong and Vierling (2000, 2001) |
| | HSC70-1 | At5g02500 | *Arabidopsis thaliana* | Thermo-tolerance | 93% | Qi et al. (2011) |
| Antioxidant activity | APX1 | At1g07890 | *Arabidopsis thaliana* | Ascorbate peroxidase | 76% | Shi et al. (2001) |
| Calcium signaling | ANNAT8 | At5g12380 | *Arabidopsis thaliana* | Heat-sensing/ signaling | 61% | Cantero et al. (2006) |

**Chlorophyll Fluorescence.** Photochemical efficiency of Photosystem II (PSII) was measured with a portable fluorometer (Model OS1-FL, Opti-Sciences, Hudson, NH) by obtaining fluorescence transients from fully expanded leaves in flowering cotton and from the first two true leaves in seedling cotton in all, four cultivars grown under optimal (32/24°C) or high (38/24°C) temperatures. Electron transport rate (ETR) and photochemical efficiency (Quantum Yield = [Fm-F]/Fm, where Fm is maximal fluorescence, and F is the steady state fluorescence prior to saturation) were obtained. Measurements were taken between 12:00 to 1:00 PM, and the same position leaf was evaluated on each plant. Three points (avoiding leaf veins) were measured on each leaf. Data was exported to Microsoft Excel for statistical analysis and plotting graphs.

**Membrane Leakage.** The same leaves used for chlorophyll fluorescence were measured for membrane leakage. Three leaf discs, avoiding leaf veins, were cut out with a cork borer and placed into a petri dish filled with deionized water. Equal amounts (10 ml) of deionized $H_2O$ was added into each vial, which was then covered with plastic wrap. Samples were kept in the dark for 48 h before measuring electrical conductivity using a single probe conductivity meter (Orion DuraProbe™ Conductivity Cell, Beverly, MA). The conductivity meter was calibrated according to the manufacturer's instructions. After the conductivity measurements (R1), samples were placed in a hot water (95°C) bath for 10 min and the conductivity was measured again (R2). Percent injury was calculated by R1/R2 X 100.

**RNA Isolation.** For cotton seedling samples, total RNA was isolated and purified with the Plant RNeasy Mini Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's protocol. For cotton flower samples, the hot borate method (Pang et al., 2011) was used for RNA isolation. RNA quality and concentration were tested using Nanodrop-1000. Samples that have OD 260/280 ratio of 1.8 to 2 and OD 260/230 of $\geq$ 1.8 were selected for gene expression analysis. Then RNA samples were diluted to 25 ng/µl using the RNase-free water, and stored at -20°C.

**Gene Expression Analysis.** The expression profile of each gene was obtained through quantitative real-time polymerase chain reactions (qRT-PCR) using the SuperScript III Platinum SYBR Green One-Step qRT-PCR kit (Invitrogen, Inc., Carlsbad, CA) according to manufacturer protocols. PCRs on target genes and the reference gene, GhPP2A, using primers given in Table 2 were carried out in an optical 96-well plate in BIO-RAD CFX96 Touch™ Real-Time PCR Detection System. Cycle threshold (CT) values from each reaction were exported into Microsoft Excel, and the relative gene expression was calculated using the ΔΔCT method (Livak and Schmittgen, 2001), where fold change in gene expression is log $2^{-\Delta\Delta CT}$, in which ΔCT = CT of target gene – CT of reference gene, and ΔΔCT = ΔCT of heat-treated sample – ΔCT of control sample.

**Table 2. Primers for real-time PCR**

| Gene | Primer | Tm | GC content | Primer Length | Sequence length |
|---|---|---|---|---|---|
| HSFA2 | FP: GAAGGAAGGGCTTAACGAGG<br>RP: GCTGACGAATGAAACTGGAGA | 60.5<br>60.1 | 55%<br>45% | 20bp<br>22bp | 202bp |
| HSP101 | FP: GGAAGTGGAATCTGCGATAGC<br>RP: GATTTTGTCCCACCACTCTTTG | 61.2<br>60.1 | 52%<br>45% | 21bp<br>22bp | 200bp |
| GHSP26 | FP: TCCTTTTGGTTTGTTGGACCC<br>RP: ACCTTCACATCCTCTTTCGTCA | 59.5<br>60.1 | 48%<br>45% | 21bp<br>22bp | 213bp |
| HSP3 | FP: AGAAAAGTTGACCCTGACCGC<br>RP:AACCTCCTCTTCGAGACCAAAC | 61.2<br>62.1 | 52%<br>50% | 21bp<br>22bp | 186bp |
| APX1 | FP: GGCACTCAGCTGGAACTTTTG<br>RP: AGCGTAGGTGAGGTTAGGGAA | 61.2<br>58.7 | 52% | 21bp | 163bp |
| HSC70-1 | FP:TTGTTACCGTCCCTGCATACTT<br>RP: GACATCAAAAGTACCGCCACC | 60.1<br>61.2 | 45%<br>52% | 22bp<br>21bp | 200bp |
| ANNAT8 | FP:CAGGATCTTGAGTACAAGGAGCA<br>RP: GTAAGTGCATCCTCATCGGTTC | 60.8<br>60.9 | 48%<br>50% | 23bp<br>22bp | 225bp |
| GhPP2A1 | FP: GATCCTTGTGGAGGAGTGGA<br>RP: GCGAAACAGTTCGACGAGAT | 60.5<br>58.4 | 55%<br>50% | 20bp<br>20bp | 100bp |

**Data Analysis.** Physiological measurements (quantum yield and membrane injury) were done on randomized plants with two replications (separate growth chambers) consisting of five plants of each genotype. The seedlings were analyzed in 2011 and flowering plants in 2012. The physiological parameters of the four cultivars grown under high and normal day/night temperature regimes were compared (from different plants) using Student's t-test ($\alpha = 0.05$). Statistical analysis was performed using JMP IN 7.0 software (SAS Institute, Cary, NC). Gene expression analysis was done on randomized plants representing three plants for each genotype for each treatment. The CT values were exported in Microsoft Excel to calculate fold change in gene expression and the standard error.

## RESULTS AND DISCUSSION

**Effect of High Temperature on Chlorophyll Function and Membrane Leakage.** The cultivars were first tested for their predicted heat response by evaluating the effect of the long term (1 wk) high day temperature (38/24°C day/night) on photosynthesis, and membrane leakage in the leaves of seedlings and flowering plants. No significant difference was found in the quantum yield [(Fm-F)/Fm] in the illuminated leaves of the seedlings or flowering plants of the four genotypes (Fig. 1a, b). The average quantum yield in the first true leaves of seedlings and the fully expanded leaves of the flowering plants were found to be between 0.65 to 0.72 and 0.7 to 0.79, respectively (Fig. 1a, b). This observation agrees with previous studies that found a slight or no effect of temperature below 40°C on the PSII in diverse cotton cultivars (Haldimann and Feller, 2004, 2005; Snider et al., 2010), suggesting that the decline in the net photosynthesis due to high temperature in plants could be due to the effect on other components of the photosynthesis apparatus (Snider et al., 2010).

The same leaves in which chlorophyll fluorescence was measured were subjected to a membrane leakage test. The integrity of plasma membrane is correlated directly with electrolyte leakage, which increases during heat stress (Hasanuzzaman et al., 2013; Pretorius et al., 2012). After analyzing multiple biochemical effects of heat stress on the heat-tolerant cultivar VH260 and heat-sensitive cultivar ST4288, Pretorius et al. (2012) reported that membrane leakage is the most reliable indicator of heat stress in cotton. Bibi et al. (2008) also found membrane leakage to be a reliable indicator of heat-stress tolerance in cotton. We observed that in the two heat-tolerant cultivars, VH260 and MNH456, membrane leakage increased somewhat in the seedlings but not in the flowering plants (Fig. 1c, d). Whereas in the two heat-sensitive cultivars, ST213 and ST4288, a marked increase in

membrane leakage was observed in both seedlings and flowering plants exposed to 38/24°C for 1 wk (Fig. 1c, d). These results confirm heat tolerance in VH260 and MNH456 plants, and indicate the presence of specific mechanisms that protect the plants from significant membrane injury at high temperature (38°C), whereas the heat-sensitive cultivars, ST213 and ST4288, experience significantly higher membrane injury due to heat stress. Of the two heat-sensitive cultivars, ST4288, a modern cultivar, showed higher membrane leakage upon heat stress than the obsolete cultivar ST218. This observation corroborates with the findings of Brown and Oosterhuis (2005), who also found higher membrane leakage in the modern cultivars (SureGrow 747 and ST474) compared to obsolete cultivars of cotton (ST213 and Deltapine 16) at 38°C.

**Identification of Cotton Orthologs of Heat-Tolerance Genes.** The molecular basis of heat-stress tolerance in cotton is not well studied, although many genes associated with heat stress have been identified in *Arabidopsis* (Kotak et al., 2007; Qu et al., 2013). Therefore, in this study, first the cotton orthologs of *Arabidopsis* heat-stress genes were identified based on DNA sequence homology with cotton expressed-sequence tags (ESTs). As a result, six *Arabidopsis* genes and one *Gossypium arboreum* L. gene, were selected. These genes included HSPs, HSFs, and general stress protein genes (Table 1). The HSPs and HSFs play important roles in heat-tolerance in plants as determined by overexpression or knockout of these genes in *Arabidopsis thaliana* (L.) Heynh. or other plants (Hong et al., 2003; Katiyar-Agrawal et al., 2003; Lee and Schöffl, 1996; Malik et al., 1999).

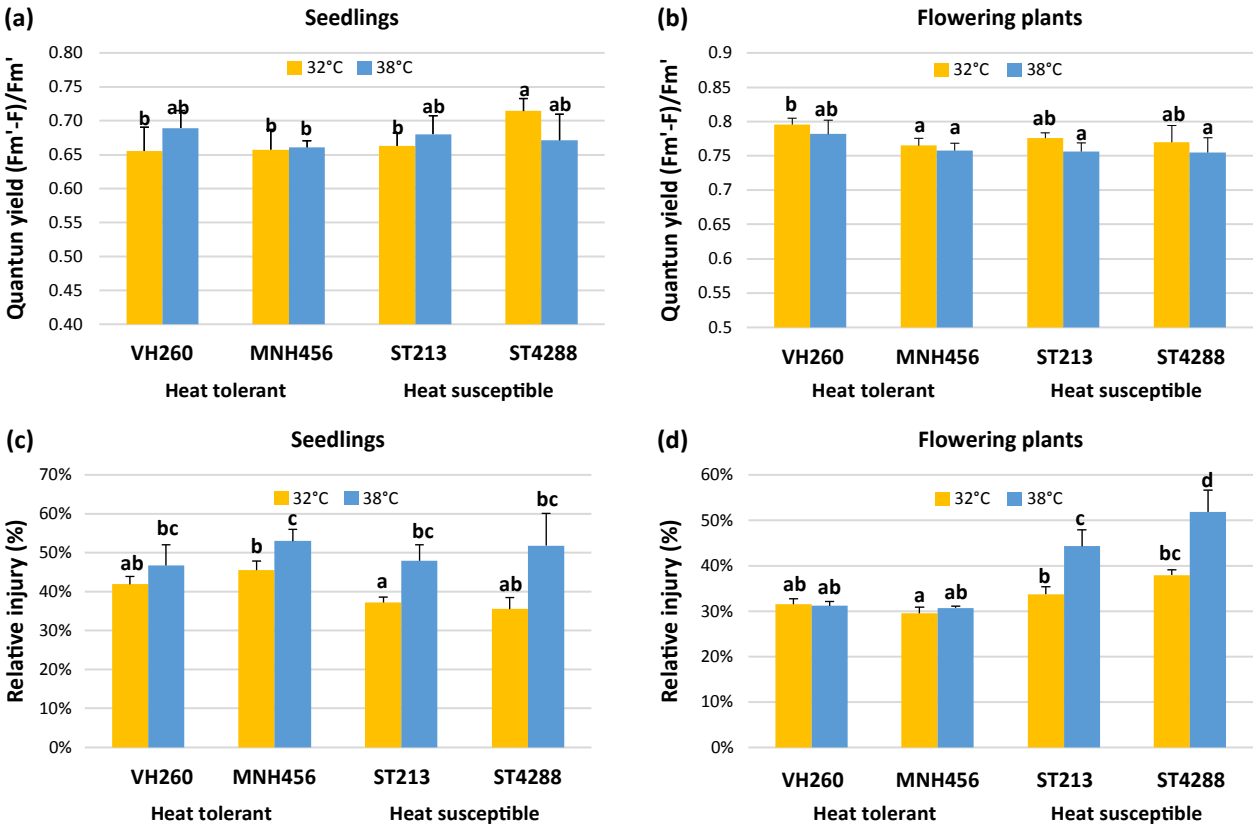


**Fig. 1. Effect of daytime heat stress on cotton physiology. (a, b) Quantum Yield in heat-tolerant (VH260 and MNH456) and heat-sensitive (ST213 and ST4288) *G. hirsutum* seedlings (a) and flowering plants (b) exposed to optimal (32/24°C; light vertical bar) or high (38/24°C; dark vertical bar) day temperatures. (c, d) Relative membrane injury as measured by electrolyte leakage in heat-tolerant (VH260 and MNH456) and heat-sensitive (ST213 and ST4288) *G. hirsutum* seedlings (c) and flowering plants (d) upon exposure to optimal (32/24°C; light vertical bar) or high (38/24°C; dark vertical bar) day temperatures. All values are means ± standard error (n = 5) of two experiments. Error bars show SD with significant differences (Student's t-test; $p < 0.1$) indicated by small letters.**

The heat-shock transcription factors, HSFA1b and HSFA2, are among the dominant heat stress genes that regulate redox homeostasis and heat-stress response network involving HSPs (Liu and Charng, 2013; Nguyen et al., 2015; Sugio et al., 2009). A large family of HSPs is found in plants that play important roles in acquired heat tolerance, some of which are critical for growth and development of plants in normal conditions. HSP101 and HSP70/HSC70-1 are the major HSPs that are induced by heat to generate heat tolerance. The overexpression or suppression of these genes in *Arabidopsis* resulted in increased heat tolerance and heat sensitivity, respectively (Hong and Vierling, 2000, 2001; Lee and Schöffl, 1996; Queitsch et al., 2000; Sung and Guy, 2003). Suppression/overexpression of HSP101 did not cause any growth abnormalities, however HSC70-1 suppression lines were nonviable, whereas overexpression lines were associated with severe growth aberrations (Sung and Guy, 2003). The HSP, GHSP26, was identified in the tropical tree cotton, *G. arboretum*, as the induced HSP in response to dehydration and found to confer drought tolerance in *G. hirsutum* (Maqbool et al., 2007, 2010). The final two genes are general stress response genes and found universally in plant kingdom. Ascorbate peroxidase (APX) provides antioxidant activity through conversion of $H_2O_2$, a potentially damaging oxidant, to $H_2O$, and ANNAT8 is a Ca++-dependent, membrane-bound protein involved in sensing the stress environment and signaling of cellular defense responses. To study the expression of these genes in response to heat in cotton, primers were designed for real-time quantitative PCR (qRT-PCR) analysis (Table 2).

**Gene Expression Analysis.** Two separate experiments were conducted to evaluate gene expression of the selected heat-stress response genes in the seedlings (first true leaves) and flowers (ovary). The first two true leaves (seedlings) and the first day flowers (flowering plants), were collected at 0, 1, 2, 3 h or d, respectively, after heat treatment for RNA isolation and gene expression analysis. All genes except, GHSP26 and ANNAT8, were analyzed in both tissue types: leaves and ovaries. GHSP26 induction by heat in the leaves, and Ca++ spiking (ANNAT8 function) in cotton flowers in response to heat are considered the potential protective mechanisms in plants (Maqbool et al., 2007; Snider et al., 2009). Therefore, these genes were analyzed in the specific tissue type. The induction of heat-shock factor gene (HSFA1b or HSFA2) in the leaves and ovaries of VH260 and MNH456 was rapid and a two to five order of magnitude higher than that of ST213 and ST4288 (Fig. 2a, b), making it one of the most discerning genes between heat-tolerant and heat-sensitive lines. GHSP26, on the other hand, was found to be induced in the leaves of all four cultivars, although at a lower level in ST4288, the modern cultivar. The induction levels of GHSP26 were similar in VH260, MNH456, and the obsolete cultivar, ST213 (Fig. 2c). These observations corroborate with the findings that obsolete cotton cultivars display lower heat-induced membrane damage than the modern cultivars; however, GHSP26 does not appear to be a critical part of the acquired heat-tolerance mechanism in these cotton lines.

HSP101 and HSC70 also were found to be expressed differentially between heat-tolerant and heat-sensitive cultivars (Fig. 3). HSP101 was induced rapidly in the seedlings and ovaries upon heat treatment in VH260 and MNH456. In contrast, its expression in ST213 and ST4288 lowered as compared to the untreated tissue (0 h/d) (Fig. 3a, b). Similarly, HSC70 induced rapidly upon heat treatment in VH260 and MNH456 seedlings but not in the ST213 and ST4288 seedlings (Fig. 3c). Notably, the HSC70 induction was found in the ovaries of only VH260 (Fig. 3d), indicating its contribution in heat tolerance of this highly tolerant cultivar. The induction of the antioxidant mechanism as indicated by APX activity in response to heat was again clearly observed in VH260, whereas other cultivars showed either none or slight induction (Fig. 4a, b). Finally, the induction of heat-sensing mechanism as indicated by ANNAT8 ortholog expression was observed only in the heat-tolerant VH260 and MNH456 cultivars; however, MNH456 and not VH260 showed more rapid induction (Fig. 4c). The two heat-sensitive cultivars, on the other hand, showed no significant change in ANNAT8 ortholog expression during 3 d of heat exposure (Fig. 4c). These results indicate that timely heat sensing and signaling of heat response is important for conferring acquired heat-tolerance phenotype in these cotton lines.

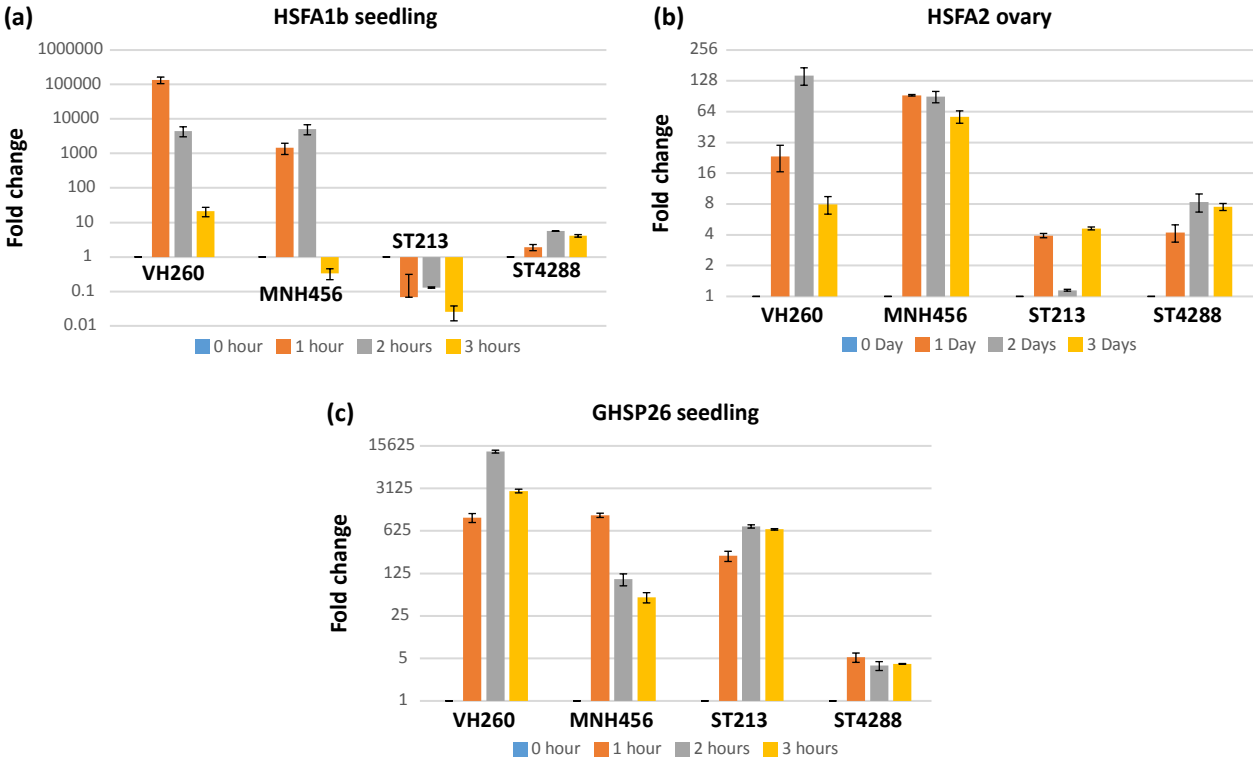


**Fig. 2. Fold change (log scale) in gene expression of heat shock factor, *HSFA1b* and *HSF2* (a, b), and heat-shock protein, *GHSP26* (c), in response to heat (38°C) exposure in heat-tolerant (VH260 & MNH456) and heat-sensitive (ST213 & ST4288) *G. hirsutum* seedlings or flowering plants. Each bar is relative to the control (0 h), which is designated a value of 1. All values are means ± standard error (n = 3).**

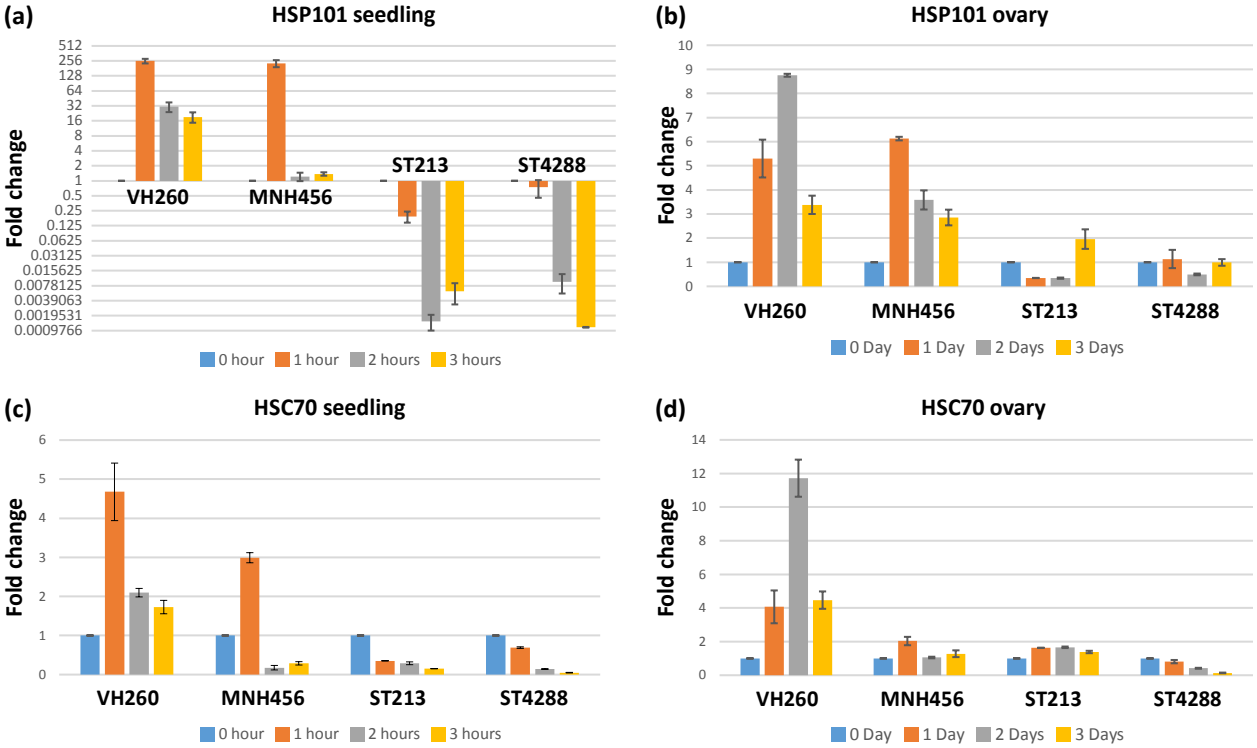


**Fig. 3. Fold change in the expression of heat shock protein genes, *HSP101* and *HSC70*, in response to heat (38°C) exposure (1-3 h) in heat-tolerant (VH260 & MNH456) and heat-sensitive (ST213 & ST43288) *G. hirsutum* seedlings (a, c) or flowering plants (b, d). Graphs are plotted in log (a) or linear (b-d) scale. Each bar is relative to the control (0 h), which is designated a value of 1. All values are means ± standard error (n = 3).**

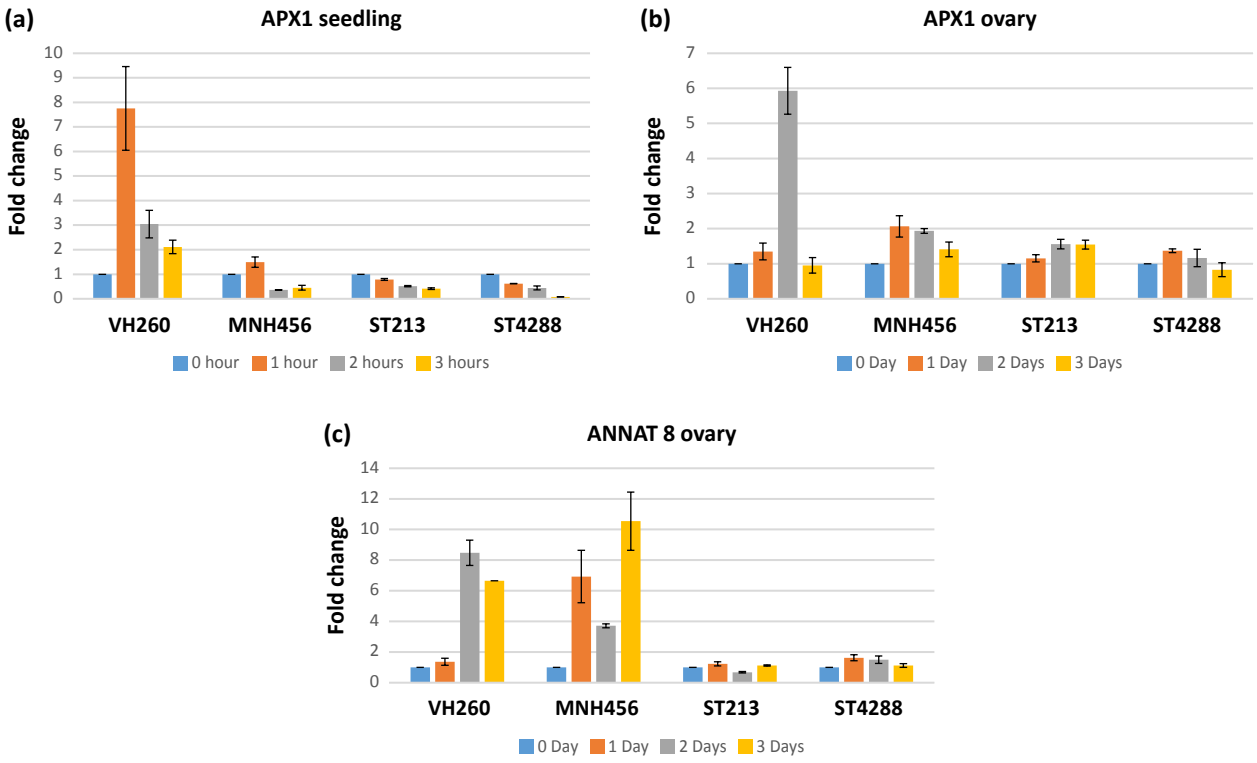


**Fig. 4. Fold change in the expression of *APX1* and *ANNAT8* genes in response to heat (38°C) exposure (1-3 h) in heat-tolerant (VH260 & MNH456) and heat-sensitive (ST213 & ST4288) *G. hirsutum* seedlings (a) and flowering plants (b, c) exposed to optimal temperature (32/24°C; black vertical bar) and high temperature (38/24°C; gray vertical bars) for 1, 2, and 3 h. Each bar is relative to the control (0 h), which is designated a value of 1. All values are means ± standard error (n = 3).**

## CONCLUSIONS

Durable heat tolerance in cotton is conferred by timely induction of multiple protective mechanisms leading to minimized electrolyte leakage and cellular damage as observed in the flowering plants of VH260 and MNH456. Due to the onset of summer season, flowering plants face higher threat of heat exposure and yield losses from heat stress on reproductive organs. Hence, proper functioning of heat-tolerance mechanisms in the flowers of cotton plants is important in boll retention and minimizing yield losses (Oosterhuis and Snider, 2011). Clearly, the HSF-HSP mechanism is important for maintaining the secondary structures of proteins, and rapid sensing of heat also is critical to induce the protective mechanisms. Finally, antioxidant protection appears to play an additive role in enhancing heat-tolerance capacity of cotton cultivars such as VH260, which is an excellent source of heat-tolerance genes for breeding and genetic engineering.

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