Cell Stress and Chaperones (2021) 26:835–843
https://doi.org/10.1007/s12192-021-01224-2

ORIGINAL PAPER

Transcriptional regulation of small heat shock protein genes by heat shock factor 1 (*HSF1*) in *Liriomyza trifolii* under heat stress

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Received: 3 April 2021 / Revised: 26 June 2021 / Accepted: 27 July 2021 / Published online: 2 August 2021

Abstract

Small heat shock proteins (sHSPs) function as molecular chaperones in multiple physiological processes and are active during thermal stress. *sHSP* expression is controlled by heat shock transcription factor (HSF); however, few studies have been conducted on HSF in agricultural pests. *Liriomyza trifolii* is an introduced insect pest of horticultural and vegetable crops in China. In this study, the master regulator, *HSF1*, was cloned and characterized from *L. trifolii*, and the expression levels of *HSF1* and five *sHSPs* were studied during heat stress. *HSF1* expression in *L. trifolii* generally decreased with rising temperatures, whereas expression of the five *sHSPs* showed an increasing trend that correlated with elevated temperatures. All five *sHSPs* and *HSF1* showed an upward trend in expression with exposure to 40 ℃ without a recovery period. When a recovery period was incorporated after thermal stress, the expression patterns of *HSF1* and *sHSPs* in *L. trifolii* exposed to 40 °C was significantly lower than expression with no recovery period. To elucidate potential interactions between *HSF1* and *sHSPs*, double-stranded RNA was synthesized to knock down *HSF1* in *L. trifolii* by RNA interference. The knockdown of *HSF1* by RNAi decreased the survival rate and expression of *HSP19.5*, *HSP20.8*, and *HSP21.3* during high-temperature stress. This study expands our understanding of *HSF1*-regulated gene expression in *L. trifolii* exposed to heat stress.

Keywords *Liriomyza trifolii* · *HSF1* · *sHSPs* · Heat stress · RNAi

Introduction

The leafminer fly, *Liriomyza trifolii* (Burgess), is an invasive, polyphagous pest that damages agricultural and horticultural crops worldwide (Spencer 1973). *L. trifolii* larvae form tunnels in foliage, and adults pierce leaf tissue for feeding and oviposition (Johnson et al. 1983; Parrella et al. 1985; Reitz et al. 1999). *L. trifolii* was first reported in mainland China in 2005 after the earlier invasion of *L. sativae* and *L. huidobrensis* (Wang et al. 2007). These three *Liriomyza* spp. are regarded as the most serious leafminer flies in the field (Wen et al. 1996; Kang et al. 2009; Xiang et al. 2012; Wan and Yang 2016), and multiple reports have documented interspecific competition among *Liriomyza* spp. (Gao et al. 2017). Because of its strong competitiveness, *L. trifolii* supplanted *L. sativae* in regions of southern China and has emerged as the predominant species (Wen et al. 1998; Wang et al. 2007; Wan and Yang 2016; Gao et al. 2017; Chang et al. 2017a, 2020a).

Temperature has played a major role in the development and distribution of *Liriomyza* spp. (Kang et al. 2009). Minor variations in thermotolerance were shown to perturb the competitive balance among congener species (Kang et al. 2009; Wang et al. 2014a, b). Several studies have investigated the response of *Liriomyza* spp. to temperature and thermally regulated interspecific competition (Reitz and Trumble 2002; Abe and Tokumaru 2008; Wang et al. 2014a, b). Genes encoding heat shock proteins (HSPs) have been identified and compared among three *Liriomyza* spp. (Huang and Kang 2007; Chang et al. 2017a). HSPs function as molecular chaperones (Gehring and Wehner 1995; Johnston et al. 1998), and their overproduction is a well-established indicator of insect tolerance to thermal stress (Feder and Hofmann 1999; Hu et al. 2014). Among HSPs, the small heat shock protein (sHSP) family has the largest diversity in function and structure and also exhibits thermoprotective

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properties (Gehring and Wehner 1995; Franck et al. 2004). In *Chilo suppressalis*, two *sHSPs* (*CsHSP19.8* and *CsHSP24.3*) were upregulated by both low and high thermal stress (Lu et al. 2014; Pan et al. 2017). In other studies, expression levels of *sHSP* genes in the Eastern spruce budworm (Quan et al. 2017), western flower thrips (Wang et al. 2014a, b), and the oriental fruit fly (Dou et al. 2017) were significantly upregulated during temperature stress. Similarly, *sHSPs* in closely related *Liriomyza* spp. were induced by high- and low-temperature stress and exhibited different expression patterns (Huang and Kang 2007; Chang et al. 2019).

The expression of HSPs is controlled by the heat shock transcription factor (HSF) family (Wiederrecht et al. 1988). Within the typical four-membered HSF family, HSF1 is regarded as the master regulator (Wu 1995; Åkerfelt et al. 2010; Gomez-Pastor et al. 2018). HSF1 is activated by phosphorylation and trimer formation and then imported to the nucleus where it activates *HSP* expression (Neef et al. 2011; Steurer et al. 2018; Kovács et al. 2019). Specifically, HSF1 binds to the heat shock element (HSE) in the promoter region of target genes; the HSE contains at least three subsequent inverted repeats (e.g. TTCnnGAAnnTTC) (Amin et al. 1988). A single form of HSF (HSF1) is present in *Drosophila melanogaster*; however, other eukaryotes may contain multiple forms of HSF (Fujikake et al. 2005; Shamovsky and Nudler 2008; Fujimoto and Nakai 2010; Anckar and Sistonen 2011; Neudegger et al. 2016). Although HSF1 has been investigated in *Drosophila* and mammals, studies on HSF1 in agricultural insect pests are lacking.

In this study, *HSF1* was cloned and characterized from *L. trifolii*, and the expression of *HSF1* and five *sHSPs* was investigated during heat stress. *HSF1* expression was silenced in *L. trifolii* using dsRNA to better understand the relationship between *HSF1* and *sHSPs*. The results provide insight regarding the role of *HSF1* in regulating *sHSP* expression during heat stress.

Materials and methods

Insects

L. trifolii was maintained at 25 ± 1 °C with a 16:8-h (light:dark) photoperiod as described previously (Chen and Kang 2002). *L. trifolii* was reared on kidney beans (*Phaseolus vulgaris*), and foliage with tunnels was collected for pupation and eclosion.

Cloning and sequence alignment of *HSF1*

Total RNA was isolated from *L. trifolii* with RNA-easy reagent (Vazyme, China, #R701), and RNA quality was assessed as described previously (Chang et al. 2019). A fragment of *HSF1* was amplified using transcriptome data (Chang et al. 2020b) and verified with gene-specific primers (Table 1). The complete *HSF1* cDNA was obtained with the SMART RACE cDNA Amplification Kit as described (Chang et al. 2019).

The *HSF1* cDNA sequence was used in BLAST searches (http://www.ncbi.nlm.gov/BLAST/) to identify homologs in other insect species. Clustal X (Thompson et al. 1997) and ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/) were used to align sequences and identify ORFs, respectively. The ExPASy Molecular Biology Server and MEGA 6.0 (Tamura et al. 2013) were used to predict HSF1 protein sequences and phylogenetic relationships with the neighbor-joining method as described previously (Chang et al. 2019). The 3D structure of the DBD domain was predicted by the SWISS-MODEL website (https://swissmodel.expasy.org/) using the *D. melanogaster* DBD domain (SMTL ID: 1hkt.1) as a template.

High-temperature treatment and expression of *HSF1* and *sHSPs* Newly emerged *L. trifolii* adults ($n = 10$) were collected and exposed to 35, 37.5, 40, 42.5, and 45 °C for 1 h in a water bath (DC-3010, Ningbo, China). Adults maintained at 25 °C served as controls. Following exposure to high temperatures, *L. trifolii* were allowed to recover at 25 °C for 1 h and were then frozen in liquid nitrogen and stored at − 80 °C. Treatments were repeated three times. For different treatment and recovery time experiments, newly emerged adults ($n = 10$; six biological replicates per temperature) were exposed for 15, 30, 60, and 120 min at 40 °C. Control adults were maintained at 25 °C. Following exposure, half of the samples (three biological replicates) were allowed to recover at 25 °C for the same length of time as the treatment (e.g. 15, 30, 60, and 120 min) and the other half (three biological replicates) were immediately frozen in liquid nitrogen, and stored at − 80 °C for RNA extraction.

Reverse transcription of total RNA (0.5 µg) was performed with the Bio-Rad iScript™ cDNA Synthesis Kit (Bio-Rad, CA, USA). qRT-PCR reactions were performed as described (Chang et al. 2017b) with a CFX-96 real-time PCR system (Bio-Rad). Gene-specific primers are shown in Table 1. Treatments contained four biological replicates, and each reaction was performed in triplicate.

dsRNA synthesis and RNAi The full-length *L. trifolii* *HSF1* was analyzed with siDirect v. 2.0 (http://sidirect2.rnai.jp/) to select potential small interfering RNA (siRNA) sequences that could be used to design dsRNA primers. A T7 promoter sequence (TAATACGACTCACTATAG GGAGA) was incorporated into the 5′ end of sense and antisense primers to facilitate transcription from both cDNA strands. The control consisted of dsRNA specific to green fluorescence protein (*GFP*) (Table 1). Purified DNA template (1.5 µg) was used to synthesize dsRNA, and products were purified with the MEGAscript™ RNAi Kit (Thermo, USA, #AM1626). The quality and quantity

Table 1 Primers used for cDNA cloning, dsRNA synthesis, and real-time quantitative PCR

Gene		Primer sequences (5′→3′)	Fragment length (bp)
cDNA cloning and full-length cDNA amplification			
HSF1	F	ACCCAAAAACTGACCACCTTAT	394
	R	ATGAGTGAAAAGCGTGAGTCCA	
	5′	GCGTGAGTCCAGTGAATCCTGCCTA	
	3′	GAGGCAGTGACTAAAGTGCTACAGG	
dsRNA synthesis			
dsHSF1	F	TAATACGACTCACTATAGGGAGA TGGACTCACGCTTTTCACTC	702
	R	TAATACGACTCACTATAGGGAGA ATAAAGACTCCTGACGCATC	
dsGFP	F	TAATACGACTCACTATAGGGAGA CCTCGTGACCACCCTGACCTAC	314
	R	TAATACGACTCACTATAGGGAGA CACCTTGATGCCGTTCTTCTGC	
qRT-PCR			
HSF1	F	ATTGTCCCTACCTGTTGGAGCAC	99
	R	GCACTTTAGTCACTGCCTCTGGT	
HSP19.5	F	GCTACCTGTTGCCTGAAAATGCT	118
	R	CTTTGGTTCATCTTTTGGTGCTG	
HSP20.8	F	AACAACTGGTGGGATGACTATGAC	144
	R	CATTTTGTGTCTGCTGCTGTTGT	
HSP21.3	F	GAAATCAATGTGAAAGTGGTGGA	175
	R	GAACCTTCAACAAGCCATCAGAT	
HSP21.7	F	CAACAGTTTGCTCCCAATGAAG	125
	R	GAGGTAGCGTCTGGAGAAGTGA	
HSP21.7b	F	CTCCAGACGCTACCTCTTGCCT	159
	R	CTTAGCAGGTTGATTGGTTTGAGT	
ACTIN	F	TTGTATTGGACTCTGGTGACGG	73
	R	GATAGCGTGAGGCAAAGCATAA	

Note: *F*, forward; *R*, reverse; *5′*, 5′ RACE primer; *3′*, 3′ RACE primer; underscored nucleotides indicate the T7 polymerase promoter sequence

of dsRNA were evaluated by gel electrophoresis and spectrophotometry, respectively.

After anesthesia with CO_2, newly emerged *L. trifolii* adults were microinjected with dsRNA (Nanoliter 2010 Injector, WPI, USA). Insects were microinjected with 5 nL (total 50 ng) of *dsGFP* or *dsHSF1* and caged on a modified 96-well plate containing a honey/water solution throughout the experiment; dead insects were removed as needed. In silencing efficiency experiments, 50 ng of *dsHSF1* was injected per adult, and insects were subjected to 40 °C for 30 min. Silencing efficiency in surviving adults was measured at 24 h post-injection by qRT-PCR. Each treatment was repeated three times. Survival rates were also calculated in treatments containing 10 injected adults; the number of viable and expired adults was recorded after exposure to 40 °C for 30 min. Each treatment was repeated seven times.

Statistical analyses

Expression levels of *HSF1* and *sHSP* genes at different temperatures and exposure times were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001), and *ACTIN* served as a reference gene (Chang et al. 2017b). One-way ANOVA (Tukey's multiple comparison) was used to detect significant differences among temperatures using SPSS v. 16.0 (SPSS, Chicago, IL, USA). For ANOVA, data were transformed for homogeneity of variances, and differences were considered statistically significant when $P < 0.05$.

For silencing efficiency, the relative abundance of target genes and survival rates were compared to the *dsGFP* control. Student's *t*-test was used to compare differences in gene expression and mortality with SPSS v. 16.0, and differences were considered significant at $P < 0.05$.

Results

Characterization and phylogenetic analysis of *L. trifolii HSF1*

HSF1 was cloned from *L. trifolii* and deposited in GenBank (accession no. MW054859); the 2013-bp ORF encodes a 75.84-kDa protein with 670 amino acids. The deduced *L. trifolii* HSF1 protein sequence contained a

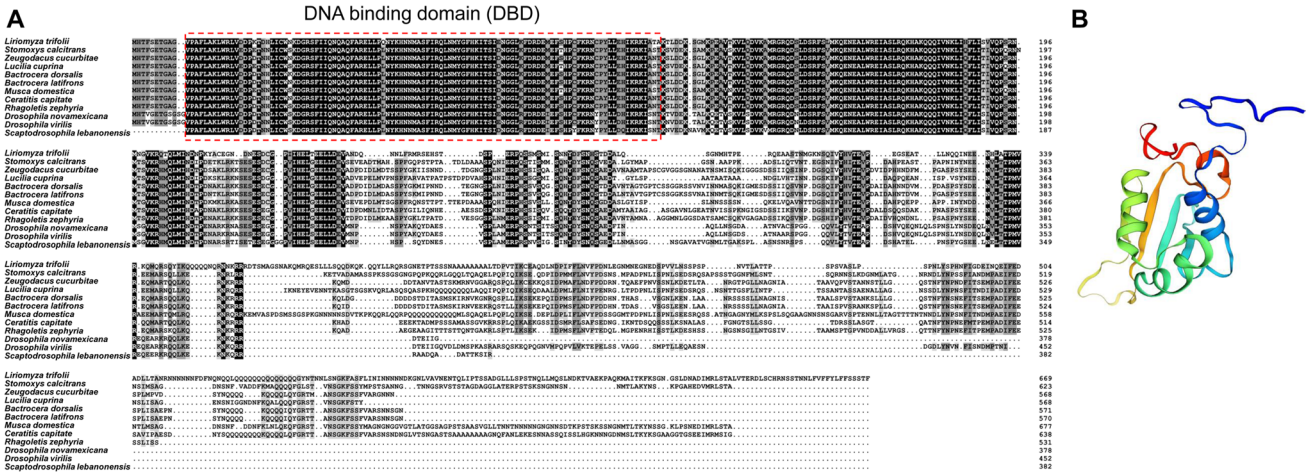

Fig. 1 Structure of HSF1 and multiple sequence alignment of HSF1 from various insect species. (A) Sequence alignment of predicted HSF1 proteins in various insect species. (B) 3D structure of HSF1 in *L. trifolii*. The deduced HSF1 protein contained a DNA-binding domain (DBD), and the 3D structure using the *D. melanogaster* DBD domain was used as a template

typical DNA-binding domain (DBD) (Fig. 1(A)). The three-dimensional (3D) structure of HSF1 from *L. trifolii* was modeled using the DBD domain in *D. melanogaster* (SMTL ID: 1hkt.1) as a template (Fig. 1(B)); HSF1 showed 83.02% sequence identity to the *D. melanogaster* orthologue. To examine relationships between HSF1s, a phylogenetic tree was generated using the neighbor-joining method; this included 12 Dipteran species and eight other insect species. HSF1 in *L. trifolii* clustered with HSF1 orthologues in other members of Diptera (Fig. 2).

Expression of *HSF1* and *sHSPs* in *L. trifolii* adults during heat stress

Expression levels of *HSF1* and five *sHSPs* (*HSP19.5*, *HSP20.8*, *HSP21.3*, *HSP21.7*, and *HSP21.7b*) were evaluated during heat stress. Compared to the control group at 25 °C, the expression levels of *HSF1* were significantly lower at 35 to 45 °C ($F_{5,17} = 7.345$, $P < 0.001$) (Fig. 3(A)). However, expression levels of the five *sHSPs* were significantly increased at elevated temperatures (*HSP19.5*: $F_{5,17} = 19.423$, $P < 0.001$; *HSP20.8*: $F_{5,17} = 36.641$,

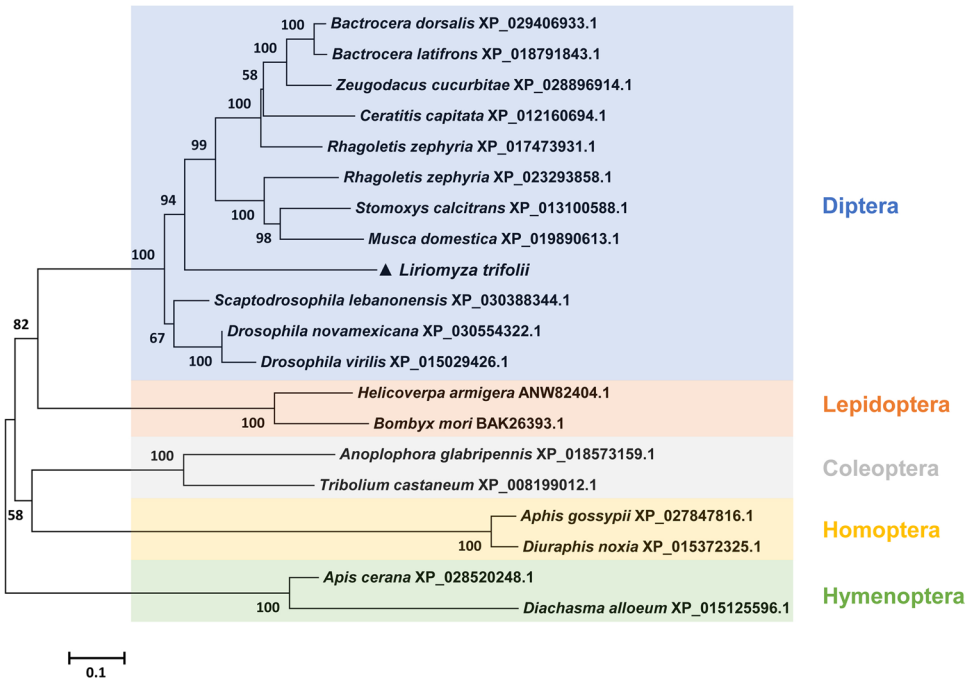

Fig. 2 Phylogenetic analysis of HSF1 from *L. trifolii* and other insects. The neighbor-joining algorithm was used to construct the tree; numbers on branches are bootstrap values obtained from 1,000 replicates (only bootstrap values > 50 are shown). HSF1 from *L. trifolii* is marked with a triangle

$P < 0.001$; *HSP21.3*: $F_{5,17} = 44.554$, $P < 0.001$; *HSP21.7*: $F_{5,17} = 29.235$, $P < 0.001$; *HSP21.7b*: $F_{5,16} = 15.980$, $P < 0.001$) (Fig. 4(A–E)). The highest expression of *sHSPs* occurred at 40 or 42.5 °C, and these expression levels were much higher than the control at 25 °C (Fig. 4(A–E)).

In experiments where *L. trifolii* was exposed to 40 °C for 15–120 min without a recovery period, expression of *HSF1* was significantly higher at 120 min vs. the control ($F_{4,10} = 10.808$, $P < 0.05$) (Fig. 3(B)). When a recovery period was incorporated into the experiment, expression of *HSF1* was significantly lower at 60 vs. 30 min ($F_{4,10} = 3.7288$, $P < 0.05$), but there was no significant difference among other treatment times (Fig. 3(C)).

Expression of *sHSPs* was also evaluated in *L. trifolii* adults exposed to 40 °C without a recovery period; expression levels for all five *sHSPs* showed significant increases beginning at 15 min and were highest at 120 min (Fig. 4(A′–E′)) (*HSP19.5*: $F_{4,10} = 44.520$, $P < 0.001$; *HSP20.8*: $F_{4,10} = 395.162$, $P < 0.001$; *HSP21.3*: $F_{4,10} = 382.432$, $P < 0.001$; *HSP21.7*: $F_{4,10} = 347.760$, $P < 0.001$; *HSP21.7b*: $F_{4,10} = 22.197$, $P < 0.001$). When a recovery period was incorporated into the experiment, the expression of the five *sHSPs* increased at different treatment times (*HSP19.5*: $F_{4,10} = 19.356$, $P < 0.001$; *HSP20.8*: $F_{4,10} = 23.762$, $P < 0.001$; *HSP21.3*: $F_{4,10} = 103.307$, $P < 0.001$; *HSP21.7*: $F_{4,10} = 45.154$, $P < 0.001$; *HSP21.7b*: $F_{4,10} = 11.365$, $P < 0.001$). The five *sHSPs* showed a consistent spike in expression levels at 30 and 120 min (Fig. 4(A″–E″)). Interestingly, there were no significant differences for *HSP21.7* and *HSP21.7b* expression in the 60 min treatment (Fig. 4(D″–E″)).

Silencing *HSF1* leads to decreased heat stress tolerance in *L. trifolii*

When insects were exposed to 40 °C for 30 min, survival was significantly reduced (~ 56.26%) in *L. trifolii* injected with *dsHSF1* as compared to *dsGFP* (Fig. 5). Quantification of mRNA by qRT-PCR revealed that *HSF1* expression was diminished by 58.65% in comparison to the *dsGFP* control ($t = 5.430$, $P < 0.05$) (Fig. 6(A)).

L. trifolii treated with *dsHSF1* were significantly impaired in transcription of *HSP19.5*, *HSP20.8*, and *HSP21.3*, which were reduced by 63.50, 34.35, and 54.36% as compared to *dsGFP*-injected controls (*HSP19.5*: $t = 5.068$, $P < 0.05$; *HSP20.8*: $t = 3.515$, $P < 0.05$; *HSP21.3*: $t = 3.082$, $P < 0.05$) (Fig. 6(B)). There were no significant differences in the expression of *HSP21.7* and *HSP21.7b* in *dsHSF1*- and *dsGFP*-treated flies (*HSP21.7*: $t = 0.836$, $P = 0.450$; *HSP21.7b*: $t = 0.813$, $P = 0.462$) (Fig. 6(B)).

Discussion

The deduced protein sequence of *HSF1* from *L. trifolii* contained the conserved DBD region, which binds to HSEs in target genes (Neudegger et al. 2016). HSF proteins use the DBD region to activate *HSPs* and other genes that encode molecular chaperones (Brunquell et al. 2016; Mahat et al. 2016; Li et al. 2017; Takii et al. 2017). The deduced 3D structures of HSF1 share typical features of the HSF1 family and phylogenetic analysis of HSF1 from *L. trifolii* revealed close relatedness with orthologous proteins in Diptera, which indicates conservation in the HSF1 family.

In general, expression of *HSF1* in *L. trifolii* decreased with rising temperatures, whereas expression of the five *sHSPs* showed an increasing trend that was concomitant with rising temperatures. These opposing transcription patterns are indicative of the temporal nature of gene expression in response to heat stress. When *HSF* is exposed to temperature stress, it shows a rapid increase in transcription, which is followed by the induction of *sHSPs*. When *sHSP* transcription is induced, *HSF1* is gradually downregulated to allow for more *HSP* expression (Nielsen et al. 2005; Anckar and Sistonen 2007). In this study, the five *sHSPs* showed

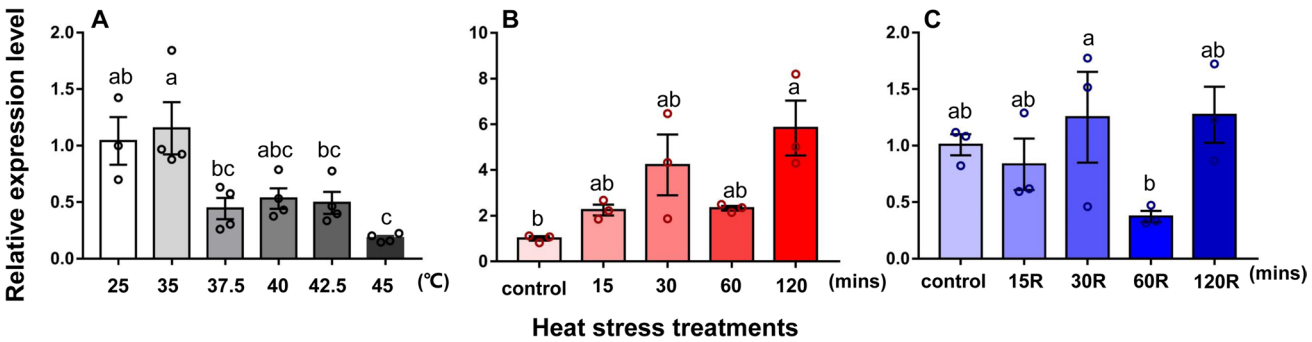

Fig. 3 Relative expression levels of *HSF1* from *L. trifolii* during heat stress. (A) Expression during different periods of high-temperature stress. (B) Expression during different periods of heat stress with no recovery period. (C) Expression during different periods of heat stress with recovery. Control adults were maintained at 25 °C. Different lowercase letters indicate significant differences between treatments. Tukey's multiple range test was used for pairwise comparison of means ($P < 0.05$)

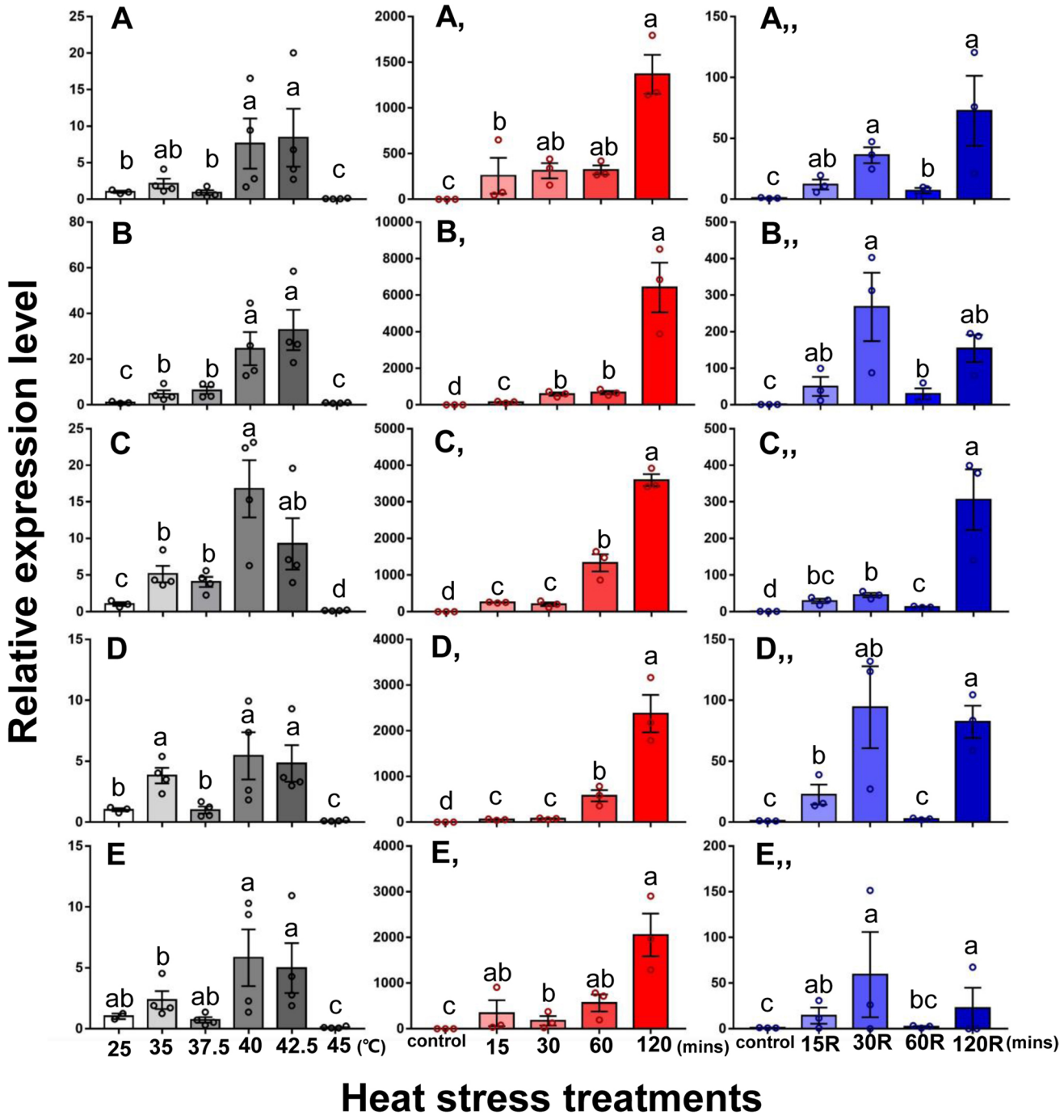

Fig. 4 Relative expression of five *LtHSPs* during different heat stress. Expression of (A) *LtHSP19.5*, (B) *LtHSP20.*8, (C) *LtHSP21.3*, (D) *LtHSP21.7*, and (E) *LtHSP21.7b* at different temperatures. Expression levels of (A′) *LtHSP19.5*, (B′) *LtHSP20.8*, (C′) *LtHSP21.3*, (D′) *LtHSP21.7*, and (E′) *LtHSP21.7b* during periods of high-temperature stress with no recovery period. Expression levels of (A″) *LtHSP19.5*, (B″) *LtHSP20.8*, (C″) *LtHSP21.3*, (D″) *LtHSP21.7*, and (E″) *LtHSP21.7b* under different periods of heat stress with recovery. Control adults were maintained at 25 °C. Different lowercase letters indicate significant differences among treatments. Tukey's multiple range test was used for pairwise comparison of means ($P < 0.05$)

varied responses to temperature, which is indicative of the cooperative effect of sHSP family members with respect to thermotolerance. In a previous study, expression of *sHSPs* in *L. trifolii* pupae showed diverse patterns during heat stress, which indicates the underlying complexity in *sHSP* evolution (Chang et al. 2019).

HSF1 was highly expressed in the 30-min recovery and 120-min "no recovery" treatments, which supports the

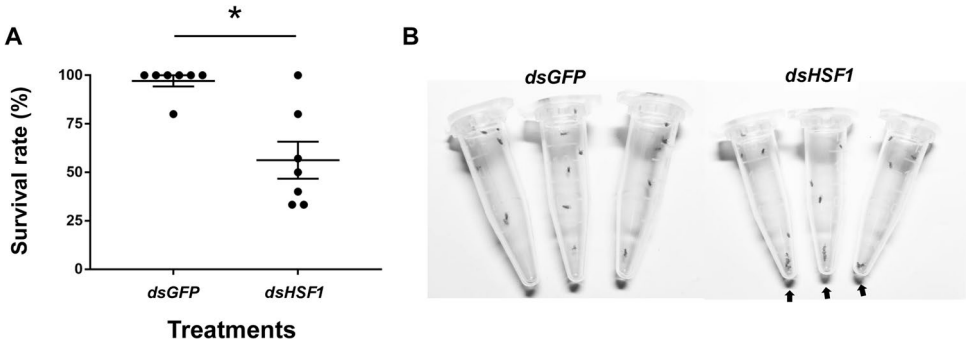

Fig. 5 RNAi-mediated knockdown of *HSF1* decreases *L. trifolii* survival. (A) Percentage of survival in insects injected with *dsHSF1* and *dsGFP* (control). (B) Insect viability after RNAi; arrows represent dead insects. Data were analyzed by Student's *t*-test, $P < 0.05$. Asterisks represent significant differences in survival of insects injected with dsHSF1 and the *dsGFP* control

contention that *HSF1* responds rapidly to thermal stress. However, it should be noted that the overall expression levels in treatments without recovery were higher than those in the recovery treatment. Furthermore, expression of the five *sHSPs* was generally higher at 30 and 120 min with a recovery period (Fig. 4(A″–E″)), which is a pattern similar to *HSF1*. In *Litopenaeus vannamei*, *HSP70* was upregulated when *HSF1* was overexpressed, which suggested that *HSF1* might function to activate the *HSP70* promoter (Yan et al. 2014). The two peaks in expression at 30 and 120 min after recovery suggest that other mechanisms of high-temperature tolerance are active. In *Frankliniella occidentalis*, *FoHSP706* expression varied at different developmental stages and treatment times (Zhang et al. 2019). In soybean seedlings, HSPs accumulated within minutes after heat shock and peaked at 1–2 h (Kimpel et al. 1990). In *D. melanogaster*, the expression of *DmHSP70Aa* peaked at 2 h following recovery after exposure to 0 °C (Colinet et al. 2010).

Suppression of *HSF1* by RNAi decreased survival and *sHSP* expression in *L. trifolii* exposed to thermal stress. The high rate of mortality indicates that *HSF1* plays a key role in thermotolerance; however, there are likely other mechanisms in place to ensure that *L. trifolii* can survive thermal stress (Guertin et al. 2010; Chang et al. 2020b). Our results show that suppression of *HSF1* decreased *HSP19.5*, *HSP20.8*, and *HSP21.3* expression, but had no significant effect on transcription of *HSP21.7* and *HSP21.7b*. These data may indicate that HSF1 binds to HSEs in *HSP19.5*, *HSP20.8*, and *HSP21.3* to regulate their expression, while *HSP21.7* and *HSP21.7b* may be regulated by other transcription factors. Interestingly, *HSP21.7* and *HSP21.7b* expression levels were not as high as the other three *sHSPs* during temperature stress. In *Artemia franciscana*, *HSF1* knockdown led to a reduction in stress tolerance proteins in diapausing embryos, which suggests that *HSF1* regulates stress-related genes (Tan and Macrae 2018). In the flea beetle, *Agasicles hygrophila*, injection of newly emerged adults with *dsAhHSF* reduced the transcription of two *HSPs* and caused reductions in egg production and survival (Jin et al. 2020). These findings indicate that further studies are needed to confirm interactions between HSF1 and sHSPs.

In summary, *HSF1* was cloned from *L. trifolii* and expressions of *HSF1* and five *sHSPs* were analyzed in response to different temperatures. To better understand the regulation of *sHSPs* expression, *dsHSF1* was generated and used to silence *HSF1* in *L. trifolii*. The knockdown of *HSF1* increased mortality in *L. trifolii* adults during heat stress and reduced stress tolerance based on the expression levels of

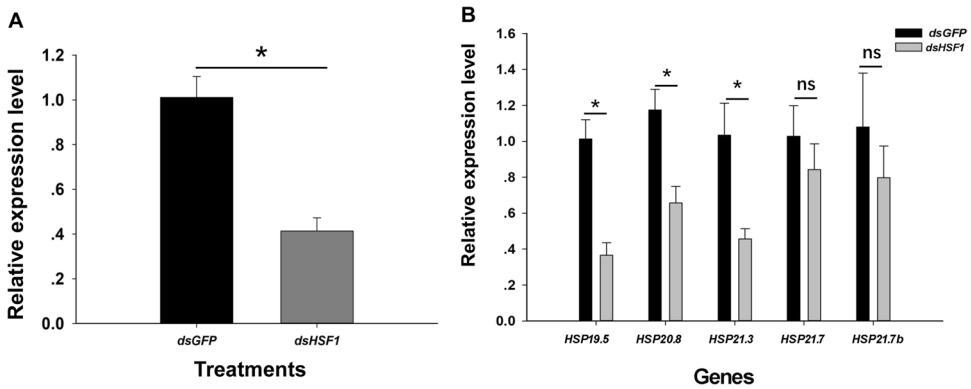

Fig. 6 *HSF1* and *sHSP* expressions in *L. trifolii* injected with *dsHSF1* and *dsGFP*. (A) Expression of *HSF1*. (B) Expression of *LtHSP19.5*, *LtHSP20.8*, *LtHSP21.3*, *LtHSP21.7*, and *LtHSP21.7b*. Data were analyzed by Student's *t*-test, $P < 0.05$. Asterisks represent significant differences between *dsGFP* and *dsHSF1*-treated insects; ns indicates no significant difference

sHSPs. The findings indicate that *HSF1* regulates the expression of stress-related genes when *L. trifolii* is exposed to high temperatures.

Acknowledgements This research was funded by the earmarked fund for Jiangsu Agricultural Industry Technology System (JATS [2020] 309), the Jiangsu Science & Technology Support Program (BE2014410), and the postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX18_2374).

Declarations

Competing interest The authors declare no competing interests.

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