Induced expression of small heat shock proteins is associated with thermotolerance in female *Laodelphax striatellus* planthoppers

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Received: 4 June 2018 / Revised: 26 October 2018 / Accepted: 2 November 2018 / Published online: 15 November 2018
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Abstract

Insects are often exposed to high temperature stress in natural environments, but the mechanisms involved in thermotolerance in many insect groups like Hemiptera are not well known. To explore possible mechanisms of thermotolerance in the hemipteran pest *Laodelphax striatellus*, which damages rice through direct feeding and viral transmission, small heat shock proteins (sHsps) implicated in thermotolerance in other insect groups were identified. The seven sHsps identified have a conserved alpha crystallin domain, a variable N-terminal region, and shared relative low identities to each other. Three of the sHsp genes (*LsHsp20.5*, *LsHsp21.5*, and *LsHsp21.6*) exhibited higher basal expression than the other four genes but showed weak or no heat-induced expression. The other four genes (*LsHsp20.1*, *LsHsp21.2*, *LsHsp21.4*, and *LsHsp22.0*) were induced up to 3306-fold by heat. Injection of dsRNA indicated that expression of these sHsps was associated with thermotolerance, and *Escherichia coli* transformed with *LsHsp21.2* and *LsHsp20.1* showed relatively higher thermotolerance. These results point to an important functional role of these sHsps for thermotolerance in *L. striatellus*.

Keywords *Laodelphax striatellus* · Thermotolerance · sHsps · Expression · Stress

Introduction

The thermotolerance of insects can be modified by heat shock protein (Hsp) synthesis (Evgen'ev et al. 2014). Although most heat shock protein research has been on Hsp70 and Hsp90, small heat shock proteins (sHsps) may also play a role in thermotolerance. Small Hsps have molecular masses in the

range of 12–43 kDa and are characterized by the presence of a conserved alpha-crystallin domain of about 90 amino acids and less conserved C-terminal and N-terminal regions (Bakthisaran et al. 2015; Mahmood et al. 2010; Nakamoto and Vigh 2007). They are involved in the maintenance of protein homeostasis (Treweek et al. 2015) and are one of the most upregulated classes of Hsps following exposure to stresses such as high or low temperatures, desiccation, oxidative stress, and starvation (Colinet et al. 2010b; King and MacRae 2015; Morrow and Tanguay 2015).

The functional role of sHsps have mostly been characterized in mammals and in model insects like *Drosophila melanogaster*, but their role in other insect groups is increasingly being investigated. The upregulated expression of sHsps has now been implicated in the thermotolerance of *Antheraea pernyi*, *Plutella xylostella*, *Chilo suppressalis*, *Spodoptera litura*, and *Cydia pomonella* (Chen and Zhang 2015; Garczynski et al. 2011; Liu et al. 2013; Lu et al. 2014; Shen et al. 2011). However, the functional role of sHsps can evolve rapidly such that related sHsps change expression to different environmental cues in related species (Zhang et al. 2015).

Few functional studies of Hsp expression have been undertaken in hemipteran planthoppers. In *Nilaparvata*

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lugens, enhanced expression of heat shock protein 90 and heat shock cognate protein 70 genes can increase resistance to temperature stress (Lu et al. 2016a, b). Rice planthoppers also increased sHsp expression under 37 °C (Huang et al. 2017), although the functional significance of these changes is unclear.

Here we investigate sHsps in the small brown planthopper (SBPH), *Laodelphax striatellus*, which damages plants by sucking plant juice and transmitting viruses (Zhang et al. 2007). The pest is found in all rice-growing areas in China (latitudes 20.03–45.8° N). High temperatures are likely to be frequently encountered by planthoppers in their natural environment. Excessively high temperatures in summer may inhibit SBPH population development in the field, with development time increasing (Wang et al. 2013) and reproduction decreasing at temperatures > 30 °C (Hachiya 1990). The diversity of sHsps in *L. striatellus* had previously been demonstrated by Huang et al. (2017), and our transcriptomic sequence analysis confirmed the presence of seven sHsp genes. To explore whether these diverse sHsps contribute to thermotolerance, planthoppers were screened for expression patterns following heat treatments, and functional analyses were undertaken based on dsRNA and bacterial studies.

Materials and methods

Insects

The SBPH population was collected in Yunnan province, China in 2001, and has been reared at 26 ± 1 °C and 60% RH in a chamber with rice seedlings since collection. For analyzing induced expression characteristics of sHsps, adults were collected and transferred to 26, 30, 33, 36, 39, 42, or 44 °C incubators for 1 h, or to a 42 °C incubator for 0, 0.25, 0.5, 1, 2, 4, 8, and 24 h and then left to recover at 26 °C for another 1 h before RNA was extracted.

Transcriptomic sequencing for screening small heat shock protein genes

The sHsps were identified from a transcriptomic database developed by Novogene Co., Ltd. Briefly, total RNA was extracted using Trizol (Invitrogen, Carlsbad, CA, USA) and quantified by 1% agarose gel electrophoresis and a NanoDrop spectrophotometer. A total of 1.5 µg RNA per sample was used as input material for sample preparations. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, Ipswich, MA, USA) following manufacturer's recommendations, and index codes were added to attribute sequences to each sample. The clustering of the index-coded samples was performed on a cBot Cluster

Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina), then the library preparations were sequenced on an Illumina HiSeq4000 platform and 150 bp paired-end reads were generated. Transcriptome assembly was accomplished by Trinity (Grabherr et al. 2011). Gene function was annotated based on databases including Nr (NCBI non-redundant protein sequences), Nt (NCBI non-redundant nucleotide sequences), Pfam (Protein family), KOG/COG (Clusters of Orthologous Groups of proteins), Swiss-Prot (a manually annotated and reviewed protein sequence database), KO (KEGG Ortholog database), and GO (Gene Ontology). The seven sHsp genes identified were annotated and resequenced for verification.

Real-time quantitative PCR

RNA was extracted from adults using the SV Total RNA Isolation System (Promega, Madison, WI, USA). Three replicate groups of six females were tested per treatment. The first-strand cDNA was synthesized from 500 ng of total RNA using PrimeScript™ RT Master Mix (Perfect Real Time) (Takara, Japan). The relative expression levels of the genes were determined with real-time quantitative PCR (qPCR) using an ADP ribosylation factor-like protein (accession number: JF728807, <https://www.ncbi.nlm.nih.gov>) as the reference gene (He et al. 2014). When the concentration of cDNA was plotted against the ct value, the slope of the amplification efficiency curve was -3.38 to -3.12 , and R^2 was 0.98–1.0. The primer sequences are shown in Table 1. qPCR was performed on an Applied Biosystems 7300 thermocycler, and reactions contained 10 µl of SYBR® Premix Ex Taq™ (Takara, Japan), 1.0 µl of cDNA, 0.4 µl of ROX reference dye (50×), and 0.4 µl of 10 µM sense and antisense primers in a 20-µl total volume. The optimized cycling program was 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s and at 60 °C for 31 s; a final disassociation stage was automatically added with 7300 System SDS software. Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). Expression levels between treatments and controls were compared with *t* tests while the basal expression among different sHsps was compared with ANOVAs followed by Tukey *b* posthoc tests.

Western blot

To test the sHsp expression of SBPH, 50 females were homogenized with RIPA buffer and the protein concentration was measured with a bicinchoninic acid (BCA) protein assay kit (Beyotime, China). The proteins were then diluted to 14 µg/µl, and 10 µl of protein extraction was loaded on 12% SDS-PAGE and transformed to a PVDF membrane (Millipore, USA) according to the

Table 1 Primer sequences for quantitative real-time PCR and dsRNA synthesis

	Genes		Nucleotide sequences (5'-3')
qPCR	<i>LsHsp20.1</i>	Forward	GATGCGTGATTTCGTGGAC
		Reverse	CTGACTGCTTCTTATTGGA
	<i>LsHsp20.5</i>	Forward	ATTGGTGGGACGACTTTGA
		Reverse	CGAGGCTTACCTGAACTTT
	<i>LsHsp21.2</i>	Forward	GTGATTGAAGGCAAACACGAAG
		Reverse	GTCTGATGGGAATATGACGCTCG
	<i>LsHsp21.4</i>	Forward	CATTTTGGATTGGGTTTGCT
		Reverse	AGGTCATTCCCCTGAGTTT
	<i>LsHsp21.5</i>	Forward	ATTCGGTCTCGGTTTGCT
		Reverse	TTGTCTTTGCGTTCCTCGT
	<i>LsHsp21.6</i>	Forward	ATGAAGAATGTAGGAGGCAGTG
		Reverse	TTGGCATGTACGAGAAAGTTTG
	<i>LsHsp22.0</i>	Forward	TTGTTGAATGATGAGTTAC
		Reverse	AGCCGTGTTTGTCTTTGCG
	Ref	Forward	TTGGACAGTATCAAGACCCATC
		Reverse	GCAGCAATGTCATCAATAAGC
dsRNA synthesis	<i>LsHsp20.1</i>	Forward	<u>TAATACGACTCACTATAGGGATGCGTGATTTCGTGGAC</u>
		Reverse	<u>TAATACGACTCACTATAGGGGCTGGTTGGTGTGTGTA</u>
	<i>LsHsp21.2</i>	Forward	<u>TAATACGACTCACTATAGGACGTGATTCGTGATTTATG</u>
		Reverse	<u>TAATACGACTCACTATAGGTGATTTGTATGTCTGATGGG</u>
	<i>LsHsp21.4</i>	Forward	<u>TAATACGACTCACTATAGGATTTGGATTGGGTTTGCTG</u>
		Reverse	<u>TAATACGACTCACTATAGGGAATGGATCGTTGA</u> GTGGTG
	<i>LsHsp22.0</i>	Forward	<u>TAATACGACTCACTATAGGATCTAATGTCGGTGCCTTTG</u>
		Reverse	<u>TAATACGACTCACTATAGGTCTCCATCTTCTCCTGTTT</u>
	<i>gfp</i>	Forward	<u>TAATACGACTCACTATAGGGGAGCGCACCATCTTCTT</u>
		Reverse	<u>TAATACGACTCACTATAGGTCTCGTTGGGGTCTTTG</u>

Underline indicates T7 polymerase promoter sequence

manufacturer's instructions. The membrane was blocked with 5% BSA in Tris-buffered saline with Tween 20 (TBST) for 1 h at 37 °C. After three washes with TBST, the membrane was incubated overnight at 4 °C with primary antibodies diluted 1:1000. The membrane was then washed and further incubated for 1 h at 37 °C with goat anti-rabbit IgG antibodies conjugated with HRP (Zoonbio Biotechnology, China) diluted 1:5000 in TBST. After four washes with TBST, the antibody-reactive bands were detected with BeyoECL star (Beyotime, China).

In order to verify the expression of recombinant sHsps, the overnight cultures were pelleted and cleaned with 200 µl double distilled water. The clean pellet was suspended by PBS (PH = 7.5, 0.2 mM) and repeated freezing and thawing three times, then disrupted by ultrasonics. The supernatant and precipitation were collected as sources of protein for SDS-PAGE. The remaining experiment followed the above protocol except that the His-tag mouse monoclonal antibody was used as the primary antibody, and the goat anti-mouse IgG antibody

conjugated with HRP was used as the second antibody. The protein from an *E. coli* BL21 transferred empty vector was used as a control.

RNAi

The RNAi method was based on Lü and Wan (2011). The DNA template of four genes (see below)—*LsHsp20.1*, *LsHsp21.2*, *LsHsp21.4*, and *LsHsp22.0*—was produced from a recombinant plasmid using primers containing a T7 promoter sequence (Table 1). Amplification reactions were conducted using a PCR Kit (Dongshen Biotech, China) protocol. The PCR products were purified using a Wizard® SV Gel and PCR Clean-up System (Promega, USA) and stored at −20 °C for dsRNA synthesis.

The dsRNA was synthesized using the T7 RiboMAX™ Express RNAi System (Promega, USA). The reaction components were 10 µl RiboMAX™ Express T7 2× buffer, 8 µl linear DNA template and 2 µl enzyme mix. The dsRNA was resuspended in nuclease-free water and analyzed by agarose

Table 2 Deduced protein sequence characteristics of sHsps from *L. striatellus*

Genes	Accession number in GenBank	Protein length (aa)	Theoretical isoelectric point	Theoretical Mw (kDa)	Location of sHsp domain profile
<i>LsHsp20.1</i>	MH830307	177	6.10	20.1	58–155
<i>LsHsp20.5</i>	KX695169	185	6.21	20.5	74–170
<i>LsHsp21.2</i>	MH830308	179	6.31	21.2	58–155
<i>LsHsp21.4</i>	MH830309	188	6.16	21.4	64–160
<i>LsHsp21.5</i>	KP994346	189	6.53	21.5	68–164
<i>LsHsp21.6</i>	MH830310	192	5.53	21.6	98–191
<i>LsHsp22.0</i>	MH830311	193	6.45	22.0	70–166

The theoretical isoelectric point and Mw was evaluated by Compute pI/Mw tool on <https://www.expasy.org/>; the predicted location of sHsps domain profile was screened by HMMER tool on <https://www.ebi.ac.uk>

gel electrophoresis. The concentration of dsRNA was measured by an Eppendorf Biophotometer Plus.

Twenty-five nanograms of dsRNA was injected into the thorax of fifth instar nymphs using a microinjector (NARISHIGE IM-9B; Nikon, Tokyo, Japan). The controls

were injected with an equal amount of dsRNA of GFP. The injected SBPH were reared on fresh rice plants at 26 ± 1 °C, with $60 \pm 10\%$ humidity, and a light–dark cycle of 14:10 h L:D until about 4 days after adults had emerged. Females were then collected for quantitative real-time PCR or heat

Fig. 1 Alignment of deduced amino acid sequences of sHsps from *Laodelphax striatellus* by BioEdit software. The location of α -crystallin domains is predicted by HMMER tool on <https://www.ebi.ac.uk> and underlined

experiments. Gene expression levels were compared between treatments and controls with *t* tests.

Adult survival test

Considering the possibility that the survival rate of the SBPH may decrease after dsRNA injection, the temperature where the survival of control SBPH was about 50% was selected as the treatment temperature. Female adults injected with dsRNA were collected into a 12 × 3-cm glass tube and transferred to the 42 °C treatment temperature for 30 min, then allowed to recover at 26 °C for 60 min. Insects were considered dead if there was no response to a gentle touch. The number of surviving insects was counted. Each treatment included 20 female adults and was repeated three times. Differences in survival between treatments and controls were compared with *t* tests.

Expression of sHsps in *E. coli* BL21

The expression of sHsps in *E. coli* followed Crack et al. (2002) with minor modifications. Briefly, full nucleotide sequences of sHsps including restriction sites (ScaI for *LsHsp21.2* and NdeI for *LsHsp20.1*, *LsHsp21.4*, and *LsHsp22* at N-terminus, and restriction sites HindIII for *LsHsp21.2* and BamHI for *LsHsp20.1*, *LsHsp21.4*, and *LsHsp22.0* at C-terminus) were created by PCR. The PCR product was then cut with the restriction endonuclease and cloned into plasmid *pET-28a-c(+)*. Eight clones were examined by restriction analysis for plasmids incorporating inserts of the appropriate size and sequenced. Finally, the plasmids that had sHsp genes were inserted into *E. coli* BL21 and resequenced.

Single colonies from the transformed cells were used to inoculate 5 ml of LB medium supplemented with Kanamycin. Liquid cultures were grown at 37 °C until they

Fig. 2 Phylogenetic tree for codon DNA sequences of sHsps by maximum likelihood. A total of 23 sHsps sequences—7 sHsps from *Laodelphax striatellus*, 10 from *Drosophila melanogaster*, and 6 from *Homo sapiens*—were used in this phylogenetic tree. The sequences from

humans were used as an outgroup. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches

reached an OD600 of 0.6. The cells were added to 0.2 mM IPTG and transferred to 30 °C overnight, then used as source for recombinant protein extraction.

Thermotolerance of recombinant *E. coli* assays

Testing the thermotolerance of *E. coli* BL21 expressing sHsps followed Crack et al. (2002). A single colony was inoculated in 2 ml of LB broth containing 30 µg/ml Kanamycin and 0.25 mM IPTG at 30 °C overnight. Culture (0.5 ml) was diluted 100 times in fresh medium supplemented with 30 µg/ml Kanamycin. Cultures were incubated at 52 °C in a water bath; 10-µl samples were removed after 0 or 30 min of heat shock, diluted in 900 µl cold LB broth, and maintained on ice. Finally, the solutions were diluted 100 times and plated on LB agar. Colonies were counted after 12 h at 37 °C, and all constructs were tested four times. Survival was expressed as the number of colonies after the heat stress treatment divided by the number of the same strain without the stress treatment and given as a percentage. Survival between recombinant *E. coli* and control were compared with *t* tests.

Results

Characteristic features of screened sHsps

According to the transcriptomic database, seven heat shock protein genes, designated *LsHsp20.1*, *LsHsp20.5*, *LsHsp21.2*, *LsHsp21.4*, *LsHsp21.5*, *LsHsp21.6*, and *LsHsp22.0* respectively, were screened in *Laodelphax striatellus*. The open reading frame of these genes contained 177–193 amino acid sequences with a 20.1–22.0-kDa molecular weight and 5.53–6.53 isoelectric point (Table 2). The alignment showed these protein sequences had a conserved alpha crystallin domain consisting of about 90 amino acids (pfam family: PF00011, <http://pfam.xfam.org/family/PF00011.20>), a variable N-terminal region, and a flexible C-terminal region and shared low identities to each other (Fig. 1). The phylogenetic tree showed that most sHsp genes from the same species were generally more similar to each other than to that from other species (Fig. 2); however, *LsHsp20.5* and *LsHsp21.6* shared more similarity with *l(2)efl* and *Hsp20*-like of *D. melanogaster*, respectively.

Induced expression profiles of sHsps by heat

The sHsp expression profiles differed between normal (26 °C) and heat stress conditions. Under normal conditions, the expression of *LsHsp21.5* was highest among the seven genes, followed by *LsHsp21.6* and *LsHsp20.5*. Expression levels of these genes were 20.66 to 231.25 times higher than the other

four genes, *LsHsp21.4*, *LsHsp22.0*, *LsHsp20.1*, and *LsHsp21.2*, which had similar expression levels (Fig. 3a).

The heat-induced profile of these genes showed the opposite pattern. After exposure to 33, 36, 39, 42, or 44 °C for 1 h, the expression of *LsHsp20.5*, *LsHsp21.5*, and *LsHsp21.6* was not induced or only slightly induced, while the expression of

Fig. 3 Expression profiles of seven sHsps of *Laodelphax striatellus* under normal (26 °C) and heat stress conditions as assessed by qPCR with SYBR Green. The values are expressed as the means $\pm$ SE. **a** Expression profiles under normal conditions. Columns labeled with different lowercase letters in **a** indicate significant differences with a posthoc test following a one way ANOVA. **b** Induced expression profiles under 26, 30, 33, 36, 39, 42 or 44 °C exposure. **c** Induced expression profiles at 42 °C for 0, 0.25, 0.5, 1, 2, 4, 8 or 24 h. The asterisk marked the significantly different expressions of sHsps between the treatment and the control by *t* tests at **b** and **c**

Fig. 4 Expression of small heat shock protein 21.5 (*LsHSP21.5*) and 21.2 (*LsHSP21.2*) under normal (26 °C) and heat stress conditions (42 °C) as assessed by western blot. Lane 1, *LsHSP21.5* after heat stress; lane 2, *LsHSP21.5* under normal conditions; lane 3, *LsHSP21.2* after heat stress; lane 4, *LsHSP21.2* under normal conditions

LsHsp20.1, *LsHsp21.2*, *LsHsp21.4*, and *LsHsp22.0* was significantly induced and generally increased with temperature (Fig. 3b). Expression levels of the four inducible genes increased over time following exposure to 42 °C, reaching maximum expression at 2 h, but then decreased (Fig. 3c).

A western blot showed that *LsHsp21.5* was expressed under normal conditions and expression was enhanced after heat treatment, while the expression of *LsHsp21.2* was only detected after heat treatment (Fig. 4).

Thermotolerance of females inhibiting sHsp expression by RNA interference

The injection of females with dsRNA of sHsps inhibited the expression of *LsHsp20.1*, *LsHsp21.2*, *LsHsp21.4*, and *LsHsp22.0* and decreased thermotolerance of SBPH (Fig. 5). The expression of *LsHsp20.1*, *LsHsp21.2*, *LsHsp21.4*, and *LsHsp22.0* was reduced by 2.32–4.99 times when compared to SBPH injected with dsRNA of GFP (Fig. 5a). Furthermore, the survival of SBPH following injection with dsRNA of sHsps after heat treatment was significantly lowered by

23.92–32.70% when compared to SBPH injected with dsRNA of GFP (Fig. 5b).

Thermotolerance of *Escherichia coli* after expression of sHsps cloned in *pET-28a-c(+)*

Cell-free extracts from *E. coli* were transformed with sHsps cDNA in *pET-28a-c(+)* and induced at 30 °C with IPTG. The supernatant extracts were detected by Western blots with antibodies to the His-tag (Fig. 6a). Immunostaining of blots showed a strong reaction with *LsHsp21.2* and *LsHsp20.1* but no reaction with *LsHsp21.4* and *LsHsp22.0*. In agreement with the Western blots, bacteria transformed with *LsHsp21.2* and *LsHsp20.1* showed greater thermotolerance than cells that had incorporated only *pET-28a-c(+)* (Fig. 6b), and the survival of BL21 was increased by 5.69% and 17.73%, respectively. These results suggest that the synthesis of sHsps proteins enhances the thermotolerance of *E. coli*.

Discussion

In this study, seven sHsp genes were identified from the transcriptome of *L. striatellus*. This was consistent with previous results from genome sequencing (Huang et al. 2017). The sHsps detected here include some that show only weak (or no) induction following heat treatment, but these additional sHsps had relatively high basal expression. This is consistent with other studies that have identified sHsps from transcriptomic or genomic data and that have found a combination of sHsps that are inducible and non-inducible under thermal stress treatments (Atungulu et al. 2007; Chen and Zhang 2015; Zhang et al. 2015). For example, ten of the sHsps in *Drosophila melanogaster* are heat inducible while the remaining two are either not heat-inducible (CG14207) or have no data available (CG43851) (Morrow and Tanguay 2015).

Fig. 5 Effects of injecting dsRNA of small heat shock proteins on the thermotolerance of *Laodelphax striatellus*. **a** The expression of *LsHsp20.1*, *LsHsp21.2*, *LsHsp21.4*, and *LsHsp22.0* of SBPH after

injection with dsRNA. **b** Mortality of SBPH at 42 °C after injection by dsRNA. Significant differences between injection with ref and target genes by *t* tests are indicated with asterisk above the bars

Fig. 6 Thermotolerance of *Escherichia coli* after expression of small heat shock protein genes cloned in *pET-28a-c(+)*. **a** The expression of small heat shock protein in transformed bacteria. Lane 1, *pET-28a-c(+)*; lane 2, *LsHsp21.2*; lane 3, *LsHsp20.1*. **b** Survival of *E. coli* transformed with

pET-28a-c(+) containing *sHsps* cDNA after heat stress (52 °C). Columns labeled with asterisk indicate significant different survival of *E. coli* between containing *sHsps* cDNA and control by *t* test

The number of sHsp genes appears to differ markedly among insects, ranging from 16 in *Bombyx mori* (Li et al. 2009) to 5 in *Chilo suppressalis* (Lu et al. 2014), with *L. striatellus* and other Lepidoptera having intermediate numbers (Chen and Zhang 2015; Li et al. 2009; Zhang et al. 2015). Some of this variation may reflect differences in the sensitivity of bioinformatic approaches used to identify sHsps and/or variation in sequence quality, but there is also likely to be a high level of gene duplication and loss in this group (Caspers et al. 1995) that influences gene number.

The sHsps we have detected show properties that are typical of small heat shock proteins, although they share relatively low identities with each other and also with the human sHsps. This situation may reflect the monophylogenetic origin of the alpha-crystallin domain on the one hand and the independent evolution of the flanking regions on the other hand, as was demonstrated in an earlier publication (Kriehuber et al., 2010). However, Zhang et al. (2015) found that sequence similarity in lepidopteran sHsps did not correlate with functional conservation in that related sHsps did not show common patterns of induction by different stresses (or diapause). It is not clear if this lack of functional constraints also extends to Hemiptera given the lack of comparative studies to date; however, sHsps do appear to have the potential to evolve rapidly and likely acquire new functions as well as being involved in routine development (Caspers et al. 1995).

Our functional analysis based on RNAi points to the four induced sHsps providing a role in protection against heat stress in *L. striatellus*. These types of experiments and other manipulations of gene expression have so far only been undertaken in a few insect species (e.g., Colinet et al. 2010a; Liu et al. 2013), but point to the important roles that sHsps can have under stressful conditions. In addition, in our experiments, recombinant *E. coli* showed a better resistance to heat when *L. striatellus* sHsps were expressed. Conversely, the thermotolerance of the SBPH was lowered following the knockdown of some sHsps.

The sHsps could be the first line of defense for cells against stress-induced protein loss and may be important for responses to crowding, anoxia, and desiccation stress in insects (King and MacRae 2015). High temperatures are likely to be frequently encountered by planthoppers in their natural environment (Zhang and Liu 2010). *L. striatellus* has two population peaks in spring and autumn in Jiangsu province, the main region where *L. striatellus* occurs in China. Maximum temperatures have exceeded 36 °C in spring and 39 °C in autumn in this region in the last 50 years (China Meteorological Data Service Center). An inducible response like the one documented here not only can protect cells against stress but also ensure that expression of the sHsps is normally very low to reduce wasteful protein synthesis, as also postulated for Hsp70 family genes (Wang et al. 2017).

In conclusion, we have identified sHsps in *L. striatellus* with clear functional roles in countering heat stress. It remains to be seen whether the sHsps are also inducible by other stresses or have roles in other processes including development and diapause (Wang et al. 2014). The current study also provides a starting point for comparative analyses of this gene family across Hemiptera.

Acknowledgements We thank Dr. Perran Ross for proofreading the revised manuscript.

Funding information This project was supported by National Natural Science Foundation of China (31572004), the Natural Science Foundation of Jiangsu Province (BK20170072), and the Food Production Engineering of China (2016YFD0300706).

References

- Atungulu E, Tanaka H, Fujita K, Yamamoto K, Sakata M, Sato E, Hara M, Yamashita T, Suzuki K (2007) A double chaperone function of the sHsp genes against heat-based environmental adversity in the soil-dwelling leaf beetles. *J Insect Biotechnol Sericol* 75(1):15–22

- Bakthisaran R, Tangirala R, Rao CM (2015) Small heat shock proteins: role in cellular functions and pathology. *Biochim Biophys Acta* 1854(4):291–319. <https://doi.org/10.1016/j.bbapap.2014.12.019>
- Caspers GJ, Leunissen JAM, de Jong WW (1995) The expanding small heat-shock protein family, and structure predictions of the conserved “ α -crystallin domain”. *J Mol Evol* 40(3):238–248. <https://doi.org/10.1007/bf00163229>
- Chen X, Zhang Y (2015) Identification of multiple small heat-shock protein genes in *Plutella xylostella* (L.) and their expression profiles in response to abiotic stresses. *Cell Stress Chaperones* 20(1):23–35. <https://doi.org/10.1007/s12192-014-0522-7>
- Colinet H, Lee SF, Hoffmann A (2010a) Knocking down expression of Hsp22 and Hsp23 by RNA interference affects recovery from chill coma in *Drosophila melanogaster*. *J Exp Biol* 213(Pt 24):4146–4150. <https://doi.org/10.1242/jeb.051003>
- Colinet H, Lee SF, Hoffmann A (2010b) Temporal expression of heat shock genes during cold stress and recovery from chill coma in adult *Drosophila melanogaster*. *FEBS J* 277(1):174–185. <https://doi.org/10.1111/j.1742-4658.2009.07470.x>
- Crack JA, Mansour M, Sun Y, MacRae TH (2002) Functional analysis of a small heat shock/ α -crystallin protein from *Artemia franciscana*. *Eur J Biochem* 269(3):933–942. <https://doi.org/10.1046/j.0014-2956.2001.02726.x>
- Evgen'ev MB, Garbuz DG, Zatssepina OG (2014) Heat shock proteins and adaptation to variable and extreme environments. Springer, Netherlands
- Garczynski SF, Unruh TR, Guédot C, Neven LG (2011) Characterization of three transcripts encoding small heat shock proteins expressed in the codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae). *Insect Sci* 18(5):473–483. <https://doi.org/10.1111/j.1744-7917.2010.01401.x>
- Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, Di Palma F, Birren B, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A (2011) Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol* 29(7):644–652. <https://doi.org/10.1038/nbt.1883>
- Hachiya K (1990) Effect of temperature on the developmental velocity of the small brown planthopper, *Laodelphax striatellus*, Fallén. *Annu Rep Soc Plant Protect N Jpn* 41:112–113
- He X, Liu C, Li Z, Zhang Z, Li G, Li F, Dong S (2014) Validation of reference genes for quantitative real-time PCR in *Laodelphax striatellus*. *J Integr Agric* 13(4):811–818. [https://doi.org/10.1016/s2095-3119\(13\)60515-8](https://doi.org/10.1016/s2095-3119(13)60515-8)
- Huang HJ, Xue J, Zhuo JC, Cheng RL, Xu HJ, Zhang CX (2017) Comparative analysis of the transcriptional responses to low and high temperatures in three rice planthopper species. *Mol Ecol* 26(10):2726–2737. <https://doi.org/10.1111/mec.14067>
- King AM, MacRae TH (2015) Insect heat shock proteins during stress and diapause. *Annu Rev Entomol* 60:59–75. <https://doi.org/10.1146/annurev-ento-011613-162107>
- Kriehuber T, Rattei T, Weinmaier T, Bepperling A, Haslbeck M, Buchner J (2010) Independent evolution of the core domain and its flanking sequences in small heat shock proteins. *FASEB J* 24(10):3633–3642
- Li ZW, Li X, Yu QY, Xiang ZH, Kishino H, Zhang Z (2009) The small heat shock protein (sHSP) genes in the silkworm, *Bombyx mori*, and comparative analysis with other insect sHSP genes. *BMC Evol Biol* 9:215. <https://doi.org/10.1186/1471-2148-9-215>
- Liu QN, Zhu BJ, Dai LS, Fu WW, Lin KZ, Liu CL (2013) Overexpression of small heat shock protein 21 protects the Chinese oak silkworm *Antheraea pernyi* against thermal stress. *J Insect Physiol* 59(8):848–854. <https://doi.org/10.1016/j.jinsphys.2013.06.001>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) method. *Methods* 25(4):402–408. <https://doi.org/10.1006/meth.2001.1262>
- Lü ZC, Wan FH (2011) Using double-stranded RNA to explore the role of heat shock protein genes in heat tolerance in *Bemisia tabaci* (Gennadius). *J Exp Biol* 214(5):764–769. <https://doi.org/10.1242/jeb.047415>
- Lu MX, Hua J, Cui YD, Du YZ (2014) Five small heat shock protein genes from *Chilo suppressalis*: characteristics of gene, genomic organization, structural analysis, and transcription profiles. *Cell Stress Chaperones* 19(1):91–104. <https://doi.org/10.1007/s12192-013-0437-8>
- Lu K, Chen X, Liu W, Zhou Q (2016a) Characterization of heat shock cognate protein 70 gene and its differential expression in response to thermal stress between two wing morphs of *Nilaparvata lugens* (Stål). *Comp Biochem Physiol A Mol Integr Physiol* 199:47–53. <https://doi.org/10.1016/j.cbpa.2016.05.009>
- Lu K, Chen X, Liu W, Zhou Q (2016b) Identification of a heat shock protein 90 gene involved in resistance to temperature stress in two wing-morphs of *Nilaparvata lugens* (Stål). *Comp Biochem Physiol A Mol Integr Physiol* 197:1–8. <https://doi.org/10.1016/j.cbpa.2016.02.019>
- Mahmood T, Safdar W, Abbasi BH, Naqvi SMS (2010) An overview on the small heat shock proteins. *Afr J Biotechnol* 9(7):927–949
- Morrow G, Tanguay RM (2015) *Drosophila* small heat shock proteins: an update on their features and functions. 8:579–606. https://doi.org/10.1007/978-3-319-16077-1_25
- Nakamoto H, Vigh L (2007) The small heat shock proteins and their clients. *Cell Mol Life Sci* 64(3):294–306. <https://doi.org/10.1007/s00018-006-6321-2>
- Shen Y, Gu J, Huang LH, Zheng SC, Liu L, Xu WH, Feng QL, Kang L (2011) Cloning and expression analysis of six small heat shock protein genes in the common cutworm, *Spodoptera litura*. *J Insect Physiol* 57(7):908–914. <https://doi.org/10.1016/j.jinsphys.2011.03.026>
- Treweek TM, Meehan S, Ecroyd H, Carver JA (2015) Small heat-shock proteins: important players in regulating cellular proteostasis. *Cell Mol Life Sci* 72(3):429–451
- Wang L, Shi P, Chen C, Xue F (2013) Effect of temperature on the development of *Laodelphax striatellus* (Homoptera: Delphacidae). *J Econ Entomol* 106(1):107–114. <https://doi.org/10.1603/ec12364>
- Wang L, Lin K, Chen C, Fu S, Xue F (2014) Diapause induction and termination in the small brown planthopper, *Laodelphax striatellus* (Hemiptera: Delphacidae). *PLoS One* 9(9):e107030. <https://doi.org/10.1371/journal.pone.0107030>
- Wang L, Shan D, Zhang Y, Liu X, Sun Y, Zhang Z, Fang J (2017) Effects of high temperature on life history traits and heat shock protein expression in chlorpyrifos-resistant *Laodelphax striatella*. *Pestic Biochem Physiol* 136:64–69. <https://doi.org/10.1016/j.pestbp.2016.08.002>
- Zhang AM, Liu XD (2010) Population characteristics of *Laodelphax striatellus* and the relationship with temperature. *Chin Bull Entomol* 47(2):326–330
- Zhang S, Li L, Wang X, Zhou G (2007) Transmission of rice stripe virus acquired from frozen infected leaves by the small brown planthopper (*Laodelphax striatellus* Fallén). *J Virol Methods* 146(1–2):359–362. <https://doi.org/10.1016/j.jviromet.2007.05.028>
- Zhang B, Zheng J, Peng Y, Liu X, Hoffmann AA, Ma CS (2015) Stress responses of small heat shock protein genes in Lepidoptera point to limited conservation of function across phylogeny. *PLoS One* 10(7):e0132700. <https://doi.org/10.1371/journal.pone.0132700>