Effects of abiotic stress on the expression of *Hsp70* genes in *Sogatella furcifera* (Horváth)

Cao Zhou¹ · Xi-bin Yang¹ · Hong Yang^{1,2} · Gui-yun Long¹ · Zhao Wang^{1,3} · Dao-chao Jin¹

Received: 22 July 2019 / Revised: 7 October 2019 / Accepted: 10 November 2019 / Published online: 26 November 2019
© Cell Stress Society International 2019

Abstract

Sogatella furcifera (Horváth), a prominent rice pest in Asia, is a typical R-strategic and highly adaptable insect. Heat shock proteins (Hsps) are highly conserved molecular chaperones regulating responses to various abiotic stresses; however, limited information is available regarding their role in responding to abiotic stress in *S. furcifera*. This study aimed to investigate the effect of abiotic stresses on the expression of *Hsp70* genes in the *S. furcifera*. Five *Hsp70* genes were isolated from *S. furcifera*, and the expression patterns at different developmental stages and temperatures, upon treatment with different insecticides and ultraviolet A (UV-A) stress, were analyzed. *Hsp70* genes were expressed at different developmental stages. *Hsp70-2*, *Hsp70-5*, and *Hsp70-6* were significantly upregulated upon heat shock at 40 °C for 30 min. *Hsp70-3* and *Hsp70-4* were significantly upregulated upon heat shock at 30 °C for 30 min. Under UV-A stress, *Hsp70-3*, *Hsp70-4*, *Hsp70-5*, and *Hsp70-6* were significantly upregulated. Conversely, *Hsp70-2* was significantly downregulated under UV-A stress. The five *Hsp70* genes were significantly downregulated in 3rd-instar nymphs on exposure to thiamethoxam, buprofezin, and avermectin at LC₁₀ and LC₂₅ concentrations. Hence, *Hsp70* genes significantly contribute to the tolerance of *S. furcifera* to temperature and UV-A stress; however, they are not involved in the response to insecticides.

Keywords *Sogatella furcifera* · Temperature · Ultraviolet A radiation · Insecticides · Heat shock protein

Heat shock proteins (Hsps) serve as molecular chaperones in response to heat shock stress and can be rapidly synthesized to cope with various environmental stressors, including cold shock, insecticides, heavy metals, and diapause (Lu et al. 2017; Rinehart et al. 2007; Shu et al. 2011). Hsps can be divided into five families based on molecular weight and homology: Hsp90, Hsp70, Hsp60, Hsp40, and small Hsps

(Feder and Hofmann 1999). The Hsp70 family is further divided into two subgroups based on their response patterns to various stimuli: stress-inducible (Hsp70) and constitutively expressed (Hsc70) proteins (Cheng et al. 2016; Shim and Lee 2015; Sun et al. 2016).

Recent studies have reported that Hsps are induced by various stresses including heat shock, cold shock, hypoxia, dryness, starvation, heavy metal exposure, and insecticide exposure (King and MacRae 2015; Koo et al. 2015; Lu et al. 2016b; Sun et al. 2016; Tang et al. 2015; Tungjitwitayakul et al. 2016; Yang et al. 2016). Hsp induction would help insects adapt to high-temperature environments owing to global warming. Hsp70 overexpression in numerous insects including *Laodelphax striatellus* (Wang et al. 2017), *Nilaparvata lugens* (Lu et al. 2017), and *Rhopalosiphum padi* (Li et al. 2017) leads to enhanced heat tolerance. In addition to environmental temperature, insecticide stress is another important response factor among insects. Sublethal concentrations of beta-cypermethrin (LC₁₀ and LC₃₀) can upregulate *RpHsp70-1* and *RpHsp70-2* in *R. padi* (Li et al. 2017). *Cydia pomonella* was exposed to acetamiprid, methomyl, carbaryl, and cypermethrin for 36 h, and *CpHSP70-2* expression

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s12192-019-01053-4>) contains supplementary material, which is available to authorized users.

✉ Hong Yang
axyridis@163.com

¹ Institute of Entomology, Provincial Key Laboratory for Agricultural Pest Management of Mountainous Regions, Guizhou University, Guiyang 550025, People's Republic of China

² College of Tobacco Science, Guizhou University, Guiyang 550025, People's Republic of China

³ College of Environment and Life Sciences, Kaili University, Kaili 556011, People's Republic of China

was induced (Yang et al. 2016). Furthermore, stress tolerance induced by Hsp70s in insects varies depending on the stage of insect development and the type of stress (Sharma et al. 2007).

Sogatella furcifera (Horváth) is a prominent rice pest in Asia. Adult *S. furcifera* and nymphs can puncture the phloem of the rice plant, thus absorbing sap and causing serious damage to rice plants; simultaneously, *S. furcifera* propagates the southern rice black-streaked dwarf virus (Zhou et al. 2008), causing severe agricultural losses. In rice fields, *S. furcifera* are often affected by various abiotic stresses, including temperature, insecticides, and ultraviolet (UV) radiation. Insect growth, development, survival, and geographic distribution are altered by environmental factors (high temperature/low temperature, insecticides, and UV radiation) (Bale et al. 2002; Wang et al. 2014). Similar to most insects, *S. furcifera* is extremely resistant and adaptable to these abiotic stresses (Kisimoto 1976; Mu et al. 2016). However, limited information is available regarding the mechanism underlying the adaptation to abiotic stresses in *S. furcifera*. In this study, we cloned full-length complementary DNA (cDNA) of five *Hsp70* genes of *S. furcifera*. We assessed the expression of these five genes at different developmental stages and at different temperatures, and their role in the response to insecticide stress in *S. furcifera* was analyzed. Furthermore, their expression profiles were assessed under UV stress. Our findings may further the current understanding of the molecular mechanism underlying the adaptation to abiotic stresses in *S. furcifera*.

Materials and methods

Insects and insecticides

As described previously (Zhou et al. 2019), *S. furcifera* were collected from a rice field in Huaxi, Guiyang, Guizhou, China, in 2013 and maintained in the laboratory on rice seedlings at 25 ± 1 °C and $70 \pm 10\%$ relative humidity with a 16:8 h (L:D) photoperiod without insecticides. Third-instar nymphs were used in the study. Thiamethoxam (96%, technical formulation) was obtained from PFchem Co., Ltd. (Nanjing, China); abamectin (96.4%, technical formulation) was obtained from Shandong Qilu King-Phar Pharmaceutical Co., Ltd. (Shandong, China); and buprofezin (97%, technical formulation) was obtained from Guangxi Pingle Pesticide Factory (Guangxi, China).

Cloning of *Hsp70* genes

Using the *Hsp70* gene annotated in the transcriptome as a query sequence (Zhou et al. 2018), Geneious 9.0 (Kearse et al. 2012) software was used to assemble the transcriptome database of *S. furcifera*, using Primer Premier 6.0 (Premier

Biosoft International, Palo Alto, CA, USA) software–designed gene-specific primers (Table S1) to verify the sequence assembled via RT-PCR. Total RNA was extracted from ten 5th-instar nymphs with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and quantified using Thermo Scientific NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). The total cDNA was synthesized using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Dalian, China) in accordance with the manufacturer's instructions.

Sequence and phylogenetic analysis

The gene and protein sequences of all five *Hsp70* members were subjected to a homology search using NCBI-BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>). The deduced protein sequences were analyzed using DNAMAN software (version 6.0; Lynnon Biosoft, Quebec, Canada). ORFs were identified using the ORF Finder software (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>). Molecular weights and theoretical isoelectric points were calculated using the SWISS-PROT (ExPASy server) tool “Compute pI / Mw” (http://au.expasy.org/tools/pi_tool.html). Conserved domains were analyzed using PFAM (<http://pfam.xfam.org>). Thereafter, phylogenetic analysis was carried out using *Hsp70* gene sequences, using the neighbor-joining (NJ) method with 1000 bootstrap replicates, using the MEGA program package, v. 6.0 (Tamura et al. 2013).

Collection of samples at different developmental stages

To analyze the expression pattern of *Hsp70* genes, we harvested eggs, 1st instar, 2nd instar, 3rd instar, 4th-instar first day, 4th-instar second day, 5th-instar first day, 5th-instar second day, 5th-instar third day, 5th-instar fourth day, and female insects emerging at 12 h, 1 day, 2 days, 3 days, 4 days, and 5 days, amounting to 16 developmental stages in total and subjected to qRT-PCR analysis. Each sample was biologically repeated three times.

Insecticide stress treatment

The rice stem dipping method (Zhou et al. 2017) was used in the study. One-hundred 3rd-instar nymphs were transferred to and reared separately in glass tubes (300 mm high $\times$ 30 mm diameter) containing rice seedlings dipped in a sublethal concentration (LC_{10} and LC_{25}) of thiamethoxam, abamectin, and buprofezin, and rice stems treated with distilled water constituted the control. The treated insects were maintained at 25 ± 1 °C and $70 \pm 10\%$ relative humidity with a 16:8 h (L:D) photoperiod in an artificial climate box. After 48 h, 15 surviving insects were randomly selected for qRT-PCR analysis.

Each sample was biologically repeated three times. The LC₁₀ and LC₂₅ values (Table S2) of thiamethoxam, abamectin, and buprofezin against *S. furcifera* were in accordance with previously reported values (Liu et al. 2016).

Temperature stress treatment

One hundred 3rd-instar nymphs were placed in a 10-ml plastic centrifuge tube and reared in an artificial climate box at 10 °C, 20 °C, 25 °C, 30 °C, and 40 °C. Fifteen nymphs were harvested for qRT-PCR analysis at 0 min, 10 min, 30 min, and 60 min, and each treatment was administered in triplicate. Each sample was biologically repeated three times.

Induction of UV-A stress

One-hundred 3rd instar nymphs were placed in a 10-ml plastic centrifuge tube. After dark adaptation for 2 h, they were irradiated with a UV-A ultraviolet lamp (model 58B-01; wavelength 315–400 nm; Nanjing Huaqiang Electronics Co., Ltd., Nanjing, China) with a radiation intensity of 300 $\mu\text{W}/\text{cm}^2$ (Huang and Toledo 1982). Fifteen nymphs were harvested for RT-qPCR analysis at 0 min (control group CK), 15 min, 30 min, 45 min, 60 min, 75 min, 90 min, and 120 min. Each sample was biologically repeated three times.

qRT-PCR analysis

As reported previously (Zhou et al. 2019), the *Hsp70* messenger RNA (mRNA) levels under different treatment conditions were measured via qRT-PCR, using FastStart Essential DNA Green Master (Indianapolis, IN, USA) in a CFX96™ real-time quantitative PCR system (Bio-Rad, Hercules, CA, USA). The ribosomal protein L9 (RPL9) (GenBank accession number: KM885285) was considered the internal control. Specific primer pairs for each gene were designed using Primer Premier 6.0 (Premier Biosoft International, Palo Alto, CA, USA; Table S1). Each 20- μl reaction mixture comprised 1 μl of sample cDNA, 1 μl of each primer (10 μM), 7 μl of DEPC H₂O, and 10 μl of FastStart Essential DNA Green Master. The qPCR cycling parameters were as follows: 95 °C for 10 min, followed by 40 cycles at 95 °C for 30 s and 60 °C for 30 s, and melting curve generation was carried out from 65 to 95 °C. To assess reproducibility, qPCR for each sample was performed in three technical and three biological replicates. The comparative $2^{-\Delta\Delta\text{CT}}$ method (Livak and Schmittgen 2001) was used to normalize relative mRNA expression levels.

Statistical analysis

Statistical analyses were performed using SPSS (version 17.0) (SPSS, Chicago, IL, USA). All data are presented as mean $\pm$

standard error (SE) values for relative expression levels. Differences among relative expression levels of each target gene were analyzed using one-way nested analysis of variance (ANOVA) and Bonferroni test ($P < 0.05$ indicating statistical significance). Furthermore, a heatmap figure was generated using the pheatmap (Kolde 2012) R packages (v.1.0.8).

Results

Cloning and characterization of *Hsp70* genes

Using a previously described method (Zhou et al. 2019), five *Hsp70* genes were identified in accordance with the transcriptome data of the *S. furcifera* and further verified via RT-PCR (Table 1), and their designations, accession numbers, lengths, ORF sizes, theoretical isoelectric points, and molecular weights are summarized in Table 1. Structural analysis revealed three characteristic domains in all five *Hsp70* proteins (Figs. 1, 2, and 3). Meanwhile, the conserved C-terminal motif EEVD of cytoplasmic *Hsp70* genes was observed. N-terminal sequences were more conserved than C-terminal sequences on the alignment of the Hsp70-2, Hsp70-3, and Hsp70-5 (Fig. 1). The C-terminus of Hsp70-4 has a conserved endoplasmic reticulum motif “HDEL” (Fig. 2), while Hsp70-2, Hsp70-3, and Hsp70-5 have a conserved cytoplasmic motif “EEVD” (Fig. 1).

Phylogenetic analysis of *Hsp70* genes

Five *Hsp70* sequences of *S. furcifera* were subjected to a homology search with the selected species indicated in Fig. 4, and a phylogenetic tree was constructed using MEGA 6.0 software with the NJ method to analyze evolutionary relationships among the five *Hsp70* proteins of *S. furcifera* and those of other species. As shown in Fig. 4, the five *Hsp70* sequences were divided into three clusters: Hsp70 in the cytosol, endoplasmic reticulum (ER), and mitochondria. The cytosol cluster was divided into two branches: inducible Hsp70 (comprising Hsp70-2 and Hsp70-5) and Hsc70 (comprising Hsp70-3) (Fig. 4). Hsp70-4 and Hsp70-6 were clustered in endoplasmic reticulum and mitochondrial groups, respectively.

Expression of *Hsp70* genes at different developmental stages

The mRNA expression levels of five *Hsp70* genes in *S. furcifera* at different developmental stages are shown in Fig. 5 (Table S3). *Hsp70* was expressed at all developmental stages. *Hsp70-2* mRNA levels peaked during the 1st-instar nymphal stage ($P < 0.05$) and gradually decreased, being the lowest at the 5th-instar third-day stage. *Hsp70-2* mRNA expression levels were significantly higher at post-emergence

Fig. 2 Nucleotide sequence of *Hsp70-4* and the deduced sequence of *Hsp70-4* of *Sogatella furcifera*. Hsp70 family signature motifs are highlighted in red. The red dot represents the C-terminal motif KDEL

third-day post-emergence, suggesting that they potentially play similar roles in the adults and nymphal stages in *S. furcifera*. Among all developmental stages, *Hsp70-2* mRNA expression levels peaked in the 1st-instar nymph; *Hsp70-3* expression levels peaked on the third day of female emergence; *Hsp70-4*, *Hsp70-5*, and *Hsp70-6* expression levels peaked in the 5th-instar second-day nymphs; these results suggest that these *Hsp70* genes play different roles in different developmental stages of *S. furcifera*.

Expression of *Hsp70* genes under temperature stress

Expression levels of the five *Hsp70* genes under temperature stress are shown in Fig. 6 (Table S4). Under high-temperature stress, the five *Hsp70* genes were significantly upregulated. *Hsp70-2* expression levels were significantly higher at 40 °C than at other temperatures ($P < 0.05$) and increased significantly with prolonged stress ($P < 0.05$). However, *Hsp70-2* expression levels at 30 °C were not significantly different when compared with the control (at 25 °C). *Hsp70-3*,

Hsp70-4, *Hsp70-5*, and *Hsp70-6* expression levels peaked at 30 min, 30 min, 60 min, and 10 min of stress at 30 °C, respectively. However, *Hsp70-4* was significantly downregulated ($P < 0.05$) at 40 °C in comparison with the control (at 25 °C). Under low-temperature stress, only *Hsp70-3* and *Hsp70-6* were significantly upregulated ($P < 0.05$) at 20 °C for 10 min and 30 min, respectively. In contrast, *Hsp70-5* was significantly downregulated under low-temperature stress in comparison with the control (at 25 °C).

Expression of *Hsp70* genes under insecticide stress

The 3rd-instar nymphs of *S. furcifera* were exposed to insecticide stress with thiamethoxam, buprofezin, and abamectin at LC₁₀ and LC₂₅ for 48 h, and the expression levels of the five *Hsp70* genes are shown in Fig. 7. *Hsp70-2*, *Hsp70-3*, *Hsp70-4*, *Hsp70-5*, and *Hsp70-6* were significantly downregulated ($P < 0.05$) under pesticide stress, suggesting that *Hsp70-2*, *Hsp70-3*, *Hsp70-4*, *Hsp70-5*, and *Hsp70-6* could not respond to insecticide stress at LC₁₀ and LC₂₅.

Fig. 3 Nucleotide sequence of *Hsp70-6* and the deduced sequence of *Hsp70-6* from *Sogatella furcifera*. *Hsp70* family signature motifs are highlighted in red

Expression of *Hsp70* genes under UV-A stress

The expression levels of the five *Hsp70* genes of *S. furcifera* were quantified via qPCR after UV-A irradiation of 3rd-instar nymphs. The results are shown in Fig. 8 (Table S5). Compared with the control (0 min), *Hsp70-3*, *Hsp70-4*, and *Hsp70-5* expression levels peaked at 90 min of UV-A irradiation, while *Hsp70-6* expression levels peaked at 60 min of UV-A irradiation. On the contrary, UV-A irradiation significantly down-regulated ($P < 0.05$) *Hsp70-2* expression levels. After UV-A irradiation, *Hsp70-3*, *Hsp70-4*, *Hsp70-5*, and *Hsp70-6* displayed a trend of gradual upregulation, followed by downregulation.

Discussion

Insects have evolved strategies to adapt to adverse environmental factors, including extreme temperatures and insecticides. Hsps are highly conserved stress response proteins in

insects (Feder and Hofmann 1999). In the present study, five *Hsp70* genes were identified, and a comparison and analysis of their protein sequences revealed three signature sequences of the *Hsp70* family. Our results indicate that members of the *Hsp70* family are highly conserved and that their C-terminal regions are generally more distinct than their N-terminal regions. Variations in the C-terminal sequence can determine the functional specificity of individual Hsps (Luo et al. 2015). The conserved C-terminal “EEVD” motif indicates that *Hsp70-2*, *Hsp70-3*, and *Hsp70-5* are cytoplasmic homologs, and the C-terminus of *Hsp70-4* contains a conserved endoplasmic reticulum motif “HDEL” (Maheswar Rao et al. 2010). Phylogenetic analysis revealed that *Hsp70-6* is a mitochondrial

Fig. 4 Phylogenetic tree constructed on the basis of the alignment of *Hsp70*s from insects. Sequences were downloaded from the GenBank protein database. Sequence labels are indicated by the species name and GenBank accession number. The asterisk denotes the *Hsp70* sequence of *Sogatella furcifera*

Fig. 5 Expression of *Hsp70* genes at different developmental stages of *Sogatella furcifera*. Heatmaps were generated using the pheatmap package. 1st: 1st instar, 2nd: 2nd instar, 3rd: 3rd instar, 4th-1d: 4th instar first day, 4th-2d: 4th instar second day, 5th-1d: 5th instar first day, 5th-2d: 5th instar second day, 5th-3d: 5th instar third day, 5th-4d: 5th instar fourth day, Fa-12h: female emergence at 12 h, Fa-1d: female emergence at 1 day, Fa-2d: female emergence at 2 days, Fa-3d: female emergence at 3 days, Fa-4d: female emergence at 4 days, Fa-5d: female emergence at 5 days

protein. However, the mitochondrial “PEAEYEEAKK” motif was not detected in Hsp70-6 (Maheswar Rao et al. 2010). In our study, only Hsp70-3 contained a GGMP tetrapeptide (Fu et al. 2010). The function of the GGMP tetrapeptide repeat at the C-terminus of Hsp70 is currently unclear. Several researchers have suggested that whether the C-terminal of the Hsp70 family gene contains a GGMP tetrapeptide repeat is a marker for distinguishing between stress-inducible (Hsp70) and constitutively expressed proteins (Hsc70) (Wu et al. 2001; Piano et al. 2005). The results of this study are consistent with this hypothesis and further demonstrate this view. The similarity between Hsp70 and its counterparts in other insect species suggests that Hsp70 responds to environmental stress in the Hsp chaperone system.

Hsps are developmentally regulated in some insects (Sharma et al. 2007). In this study, *Hsp70-2* expression levels peaked in the first-instar nymph and gradually decreased until the 5th-instar stage, concurrent with *N. lugens* homologs (Lu et al. 2017). Furthermore, in *Tribolium castaneum*, *Hsp70III* was significantly upregulated in young larvae compared to that in old larvae (Mahroof et al. 2005). In contrast, *Hsp70-4*, *Hsp70-5*,

and *Hsp70-6* expression levels peaked at the nymphal stage on the 5th-instar second day, concurrent with *CpHSP70-2* expression levels in *Cydia pomonella*, in 5th-instar larvae (Yang et al. 2016). Our results show that *Hsp70-3* was gradually upregulated during the first-instar nymphal stage to adulthood and peaked on the third day of female emergence, concurrent with *NHsc70* in *N. lugens*, which was most upregulated in female insects (Lu et al. 2016a). The difference in *Hsp70* expression patterns at different developmental stages suggests that they potentially play different roles in protection against environmental stress at different developmental stages.

Climatic factors, particularly temperature, potentially influence the distribution and abundance of insects (Zhang and Denlinger 2010). Hsp overexpression in response to heat stress is directly associated with the acquisition of heat tolerance in insects, although these reactions are different in different insects. In this study, five *Hsp70* genes of *S. furcifera* were significantly upregulated under high- and low-temperature stress compared with the control, although the induction time and temperature were different. In particular, *Hsp70-2*

Fig. 6 Effect of temperature stress on *Hsp70* gene expression levels in *Sogatella furcifera*. Heatmaps were generated using the pheatmap package

expression levels peaked at a 40 °C temperature stress for 60 min, its expression levels being approximately 320-fold that of the control group, indicating that *Hsp70-2* plays an important role in adapting to high-temperature stress in *S. furcifera*. Similarly, *RpHsp70-1* and *RpHsp70-2* expression levels of apterous adult aphids of *R. padi* were significantly higher than those of the control (Li et al. 2017) at 36 °C. A study on *Corythucha ciliata* revealed that *CcHSP70* expression levels increased gradually with an increase in temperature and peaked at 41 °C (Ju et al. 2018). Similar results were obtained with *Leptinotarsa decemlineata* upon heat treatment (Dumas et al. 2019). In addition, the transcriptome analyses of three rice planthoppers found that a high temperature can induce the expression of the heat shock protein 70 gene (Huang et al. 2017). These results further confirmed that heat stress can induce *Hsp70* gene expression and helps insects adapt to temperature stress. Furthermore, *Hsp70-3* and *Hsp70-6* were significantly upregulated at 20 °C for 10 min and 30 min, respectively, in comparison with the control (25 °C). However, under temperature stress at 10 °C, all genes were inhibited. Similar results were obtained with *L. decemlineata*. Similar results were found in the *L. decemlineata* study. Compared to the control (maintained at 15 °C), when *L. decemlineata* were exposed to

5 °C, the expression level of the *Hsp70* gene was significantly induced; however, when *L. decemlineata* were exposed to −5 °C, the expression level of the *Hsp70* gene was significantly inhibited (Dumas et al. 2019). A study of *Apolygus lucorum* found that the expression of the *Alhsc70* gene was significantly reduced by the 4 °C treatment when compared with the control (Sun et al. 2016). However, *Hsp70* induction has not been reported in *Drosophila melanogaster* (Nielsen et al. 2005). Nonetheless, exposure to a low temperature significantly induces *Hsp70* gene expression in other insects (Paim et al. 2016; Hu et al. 2018). These studies indicate that *Hsp70* plays different roles in the adaptation to low- and high-temperature environments.

As molecular chaperones, Hsps are potentially induced upon insecticide treatment and contribute to insecticide resistance (Yoshimi et al. 2002), although these reactions differ among insect species. For example, the treatment of *R. padi* with beta-cypermethrin at LC₃₀ significantly upregulated *RpHsp70-1* and *RpHsp70-2* after 24 h and 36 h (Li et al. 2017). Treatment of *Apolygus lucorum* with cyhalothrin at LD₅₀ significantly upregulated *AlHSC70*; however, chlorpyrifos significantly downregulated *AlHSC70* (Sun et al. 2016). Furthermore, in *N. lugens*, exposure to imidacloprid (0.02 ng and 0.04 ng per female) significantly upregulated *NIHsp70*; however, this

Fig. 7 Effect of insecticide stress on the expression levels of *Hsp70* genes in *Sogatella furcifera*. Mean $\pm$ SE values determined using the $2^{-\Delta\Delta C_t}$ method are used to denote relative expression levels under different insecticide concentrations, with non-insecticide treatment (CK) as a

reference. Different letters indicate significant differences among treatments for the same duration. **a** Thiamethoxam. **b** Buprofezin. **c** Abamectin

gene was significantly downregulated with an increase in the treatment concentration (Lu et al. 2017). Moreover, a study on *L. decemlineata* reported no significant effect on *Hsp70* expression levels upon imidacloprid and chlorantraniliprole treatment (Dumas et al. 2019). Our results show that thiamethoxam, buprofezin, and abamectin treatment at LC₁₀ and LC₂₅ significantly downregulated the five *Hsp70* genes after 48 h in *S. furcifera* nymphs. These results confirm that *Hsp70* genes have different functions in response to insecticides in different insects, and their response levels are different for different insecticides. In a study of *R. padi*, it was found that after treatment of apterous aphids with beta-cypermethrin LC₁₀ and LC₃₀, the expression of *RpHsp70-1* and *RpHsp70-2* first showed inhibition, then was significantly upregulated and, finally, gradually returned to a normal level (Li et al. 2017). In a study of *N. lugens*, it was also found that the expression level of *N1Hsp70* was first induced then returned

to a normal level after each newly emerged female adult was treated with 0.04 ng per female imidacloprid (Lu et al. 2017). There was a significant time effect. In this study, we found that the expression levels of five *SfHsp70* genes were significantly inhibited after 48 h of insecticide stress. The reason for this result may be due to the time effect, and its specific reasons require further study.

For herbivorous insects directly interacting with plants, sunlight is an important source of abiotic stress (Zhao and Jones 2012). UV-A radiation constitutes the long-wavelength band of ultraviolet radiation, which causes harmful effects through the generation of reactive oxygen species (Schauen et al. 2007). This study shows that UV-A radiation can upregulate *Hsp70-3*, *Hsp70-4*, *Hsp70-5*, and *Hsp70-6*, concurrent with previous reports on *Drosophila melanogaster*, showing that UV-A radiation upregulates *DmHsp70* (Wang et al. 2014). *Hsp70-3*, *Hsp70-4*, *Hsp70-5*, and *Hsp70-6* expression levels displayed a decreasing trend

Fig. 8 Effect of UV-A stress on the expression levels of *Hsp70* genes in *Sogatella furcifera*. Heatmaps were generated using the pheatmap package

with a prolonged duration of UV-A irradiation. A similar phenomenon has been reported in *T. castaneum* and *D. melanogaster* (Sang et al. 2012; Wang et al. 2014). The reduction in Hsp70 transcription levels may have occurred because insects are exposed to UV radiation for a long period, and UV-induced cell damage exceeds the maximum tolerance restored by the upregulation of Hsp70 genes (Kim et al. 2011). Hsp70 is detrimental to normal cell growth in *Drosophila* (Feder et al. 1992), requiring a specific negative feedback regulatory mechanism for Hsp70 to balance normal cellular metabolism (Li and Duncan 1995). *Hsp70* expression levels first increase and then decrease after prolonged exposure to UV-A radiation, indicating that it may be regulated by a negative feedback regulation mechanism to prevent the accumulation of harmful substances affecting normal cell growth. Specific reasons warrant further investigation.

In this study, we identified five *Hsp70* genes in *S. furcifera* and quantified their expression levels at different developmental stages, different temperature stress, insecticide stress, and UV-A stress. Our results show that the five *Hsp70* genes were expressed at different levels at different developmental stages, different temperatures, and UV-A radiation, indicating that different *Hsp70* genes may function differently in responding to abiotic stress in *S. furcifera*. However, our results also show that under stress conditions with three insecticides, the five *Hsp70* genes were significantly downregulated, probably

because the *Hsp70* genes assessed herein are only some of the *Hsp70* genes in *S. furcifera*. Therefore, future studies are required to comprehensively assess *Hsp70* family genes through insect genomic data to further the current understanding of the role of Hsp70 in insect adaptation to environmental factors.

Funding information This study was supported by the National Natural Science Foundation of China (Grant No. 31560522 and 31960537), Provincial Key Project for Agricultural Science and Technology of Guizhou (NY20133006 and NY20103064), International Cooperation Base for Insect Evolutionary Biology and Pest Control ([2016]5802), and Graduate Education Innovation Project of Guizhou Province (Qian Jiao He YJSCXJH, No. [2018]043).

References

- Bale JS, Masters GJ, Hodkinson ID (2002) Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Glob Chang Biol* 8:1–16. <https://doi.org/10.1046/j.1365-2486.2002.00451>
- Cheng W, Li D, Wang Y, Liu Y, Zhu-Salzman K (2016) Cloning of heat shock protein genes (*hsp70*, *hsc70* and *hsp90*) and their expression in response to larval diapause and thermal stress in the wheat blossom midge, *Sitodiplosis mosellana*. *J Insect Physiol* 95:66–77. <https://doi.org/10.1016/j.jinsphys.2016.09.005>
- Dumas P, Morin MD, Boquel S, Moffat CE, Morin PJ (2019) Expression status of heat shock proteins in response to cold, heat, or insecticide exposure in the Colorado potato beetle *Leptinotarsa decemlineata*.

- Cell Stress Chaperones 4:539–547. <https://doi.org/10.1007/s12192-019-00983-3>
- Feder ME, Hofmann GE (1999) Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. *Annu Rev Physiol* 61:243–282. <https://doi.org/10.1146/annurev.physiol.61.1.243>
- Feder JH, Rossi JM, Solomon J, Solomon N, Lindquist S (1992) The consequences of expressing hsp70 in *Drosophila* cells at normal temperatures. *Genes Dev* 6:1402–1413. <https://doi.org/10.1101/gad.6.8.1402>
- Fu W, Shuai L, Yao J, Yu SH, Liu YF, Duan LD (2010) Molecular cloning and analysis of a cytosolic Hsp70 gene from *Enteromorpha prolifera* (Ulvoophyceae, Chlorophyta). *Plant Mol Biol Report* 28:430–437. <https://doi.org/10.1007/s11105-009-0170-8>
- Hu F, Ye K, Tu XF, Lu YJ, Thakur K, Wei ZJ (2018) Identification and expression analysis of four heat shock protein genes associated with thermal stress in rice weevil, *Sitophilus oryzae*. *J Asia Pac Entomol* 21:872–879. <https://doi.org/10.1016/j.aspen.2018.06.009>
- Huang YW, Toledo R (1982) Effect of high doses of high and low intensity UV irradiation on surface microbiological counts and storage-life of fish. *J Food Sci* 47(5):1667–1669. <https://doi.org/10.1111/j.1365-2621.1982.tb05007.x>
- 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>
- Ju RT, Luo QQ, Gao L, Yang J, Li B (2018) Identification of HSP70 gene in *Corythucha ciliata* and its expression profiles under laboratory and field thermal conditions. *Cell Stress Chaperones* 23:195–201. <https://doi.org/10.1007/s12192-017-0840-7>
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Kim RO, Rhee JS, Won EJ, Lee KW, Kang CM, Lee YM, Lee JS (2011) Ultraviolet B retards growth, induces oxidative stress, and modulates DNA repair-related gene and heat shock protein gene expression in the monogonot rotifer, *Brachionus* sp. *Aquat Toxicol* 101:529–539. <https://doi.org/10.1016/j.aquatox.2010.12.005>
- 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>
- Kisimoto R (1976) Synoptic weather conditions inducing long-distance immigration of planthoppers, *Sogatella furcifera* Horvath and *Nilaparvata lugens* Stal. *Ecol Entomol* 1(2):95–109. <https://doi.org/10.1111/j.1365-2311.1976.tb01210.x>
- Kolde R (2012) Pheatmap: pretty heatmaps. R package version, 61:617. <https://CRAN.R-project.org/package=pheatmap>. Accessed Aug 2019
- Koo J, Son TG, Kim SY, Lee KT (2015) Differential responses of *Apis mellifera* heat shock protein genes to heat shock, flower-thinning formulations, and imidacloprid. *J Asia Pac Entomol* 18:583–589. <https://doi.org/10.1016/j.aspen.2015.06.011>
- Li D, Duncan RF (1995) Transient acquired thermotolerance in *Drosophila*, correlated with rapid degradation of Hsp70 during recovery. *Eur J Biochem* 231:454–465. <https://doi.org/10.1111/j.1432-1033.1995.0454e.x>
- Li Y, Zhao Q, Duan X, Song C, Chen M (2017) Transcription of four *Rhopalosiphum padi* (L.) heat shock protein genes and their responses to heat stress and insecticide exposure. *Comp Biochem Physiol A Mol Integr Physiol* 205:48–57. <https://doi.org/10.1016/j.cbpa.2016.12.021>
- Liu LL, Dai RH, Yang H, Jin DC (2016) Sublethal effects of triazophos on the life table parameters of *Sogatella furcifera* (Hemiptera: Delphacidae). *Fla Entomol* 99:292–296. <https://doi.org/10.1653/024.099.0221>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25:402–408. <https://doi.org/10.1006/meth.2001.1262>
- 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 MX, Li HB, Zheng YT, Shi L, Du YZ (2016b) Identification, genomic organization and expression profiles of four heat shock protein genes in the western flower thrips, *Frankliniella occidentalis*. *J Therm Biol* 57:110–118. <https://doi.org/10.1016/j.jtherbio.2016.03.005>
- Lu K, Chen X, Liu W, Zhang Z, Wang Y, You K, Li Y, Zhang R, Zhou Q (2017) Characterization of heat shock protein 70 transcript from *Nilaparvata lugens* (Stål): its response to temperature and insecticide stresses. *Pestic Biochem Physiol* 142:102–110. <https://doi.org/10.1016/j.pestbp.2017.01.011>
- Luo S, Ahola V, Shu C, Xu C, Wang R (2015) Heat shock protein 70 gene family in the Glanville fritillary butterfly and their response to thermal stress. *Gene* 556:132–141. <https://doi.org/10.1016/j.gene.2014.11.043>
- Maheswar Rao JL, Reddy PS, Mishra RN, Gupta D, Sahal D, Tuteja N, Sopory SK, Reddy MK (2010) Thermo and pH stable ATP-independent chaperone activity of heat-inducible Hsp70 from *Pennisetum glaucum*. *Plant Signal Behav* 5:110–121. <https://doi.org/10.4161/psb.5.2.10547>
- Mahroof R, Zhu KY, Neven L, Subramanyam B, Bai J (2005) Expression patterns of three heat shock protein 70 genes among developmental stages of the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae). *Comp Biochem Physiol A Mol Integr Physiol* 141:247–256. <https://doi.org/10.1016/j.cbpb.2005.05.044>
- Mu XC, Zhang W, Wang LX, Zhang S, Zhang K, Gao CF, Wu SF (2016) Resistance monitoring and cross-resistance patterns of three rice planthoppers, *Nilaparvata lugens*, *Sogatella furcifera* and *Laodelphax striatellus* to dinotefuran in China. *Pestic Biochem Physiol* 134:8–13. <https://doi.org/10.1016/j.pestbp.2016.05.004>
- Nielsen MM, Overgaard J, Sørensen JG, Holmstrup M, Justesen J, Loeschcke V (2005) Role of HSF activation for resistance to heat, cold and high-temperature knock-down. *J Insect Physiol* 51:1320–1329. <https://doi.org/10.1016/j.jinsphys.2005.08.002>
- Paim RMM, Araujo RN, Leis M, Sant’anna MRV, Gontijo NF, Lazzari CR, Pereira MH (2016) Functional evaluation of heat shock proteins 70 (HSP70/HSC70) on *Rhodnius prolixus* (Hemiptera, Reduviidae) physiological responses associated with feeding and starvation. *Insect Biochem Mol Biol* 77:10–20. <https://doi.org/10.1016/j.ibmb.2016.07.011>
- Piano A, Franzellitti S, Tinti F, Fabbri E (2005) Sequencing and expression pattern of inducible heat shock gene products in the European flat oyster, *Ostrea edulis*. *Gene* 361:119–126. <https://doi.org/10.1016/j.gene.2005.06.034>
- Rinehart JP, Li A, Yocum GD, Hayward SA, Denlinger DL (2007) Up-regulation of heat shock proteins is essential for cold survival during insect diapause. *PNAS* 104:11130–11137. <https://doi.org/10.1073/pnas.0703538104>
- Sang W, Ma WH, Qiu L, Zhu ZH, Lei CL (2012) The involvement of heat shock protein and cytochrome P450 genes in response to UV-A exposure in the beetle *Tribolium castaneum*. *J Insect Physiol* 2012(58):830–836. <https://doi.org/10.1016/j.jinsphys.2012.03.007>
- Schauen M, Hornig-Do HT, Schomberg S, Hermann G, Wiesner RJ (2007) Mitochondrial electron transport chain activity is not involved in ultraviolet A (UVA)-induced cell death. *Free Radic Biol Med* 42:499–509. <https://doi.org/10.1016/j.freeradbiomed.2006.11.016>

- Sharma S, Rohilla MS, Tiwari PK (2007) Developmental and hyperthermia-induced expression of the heat shock proteins HSP60 and HSP70 in tissues of the housefly *Musca domestica*: an in vitro study. *Genet Mol Biol* 30:159–168. <https://doi.org/10.1590/S1415-47572007000100027>
- Shim JK, Lee KY (2015) Molecular characterization of heat shock protein 70 cognate cDNA and its upregulation after diapause termination in *Lycorma delicatula* eggs. *J Asia Pac Entomol* 18:709–714. <https://doi.org/10.1016/j.aspen.2015.08.005>
- Shu Y, Du Y, Wang J (2011) Molecular characterization and expression patterns of *Spodoptera litura* heat shock protein 70/90, and their response to zinc stress. *Comp Biochem Physiol A Mol Integr Physiol* 158:102–110. <https://doi.org/10.1016/j.aspen.2015.08.005>
- Sun Y, Zhao J, Sheng Y, Xiao YF, Zhang YJ, Bai LX, Tan Y, Xiao LB, Xu GC (2016) Identification of heat shock cognate protein 70 gene (Hsc70) of *Apolygus lucorum* and its expression in response to different temperature and pesticide stresses. *Insect Sci* 23:37–49. <https://doi.org/10.1111/1744-7917.12193>
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729. <https://doi.org/10.1093/molbev/mst197>
- Tang XT, Sun M, Lu MX, Du YZ (2015) Expression patterns of five heat shock proteins in *Sesamia inferens* (Lepidoptera: Noctuidae) during heat stress. *J Asia Pac Entomol* 18:529–533. <https://doi.org/10.1016/j.aspen.2015.07.005>
- Tungjitwitayakul J, Tatun N, Vajarasathira B, Sakurai S (2016) Effects of ultraviolet-C and microwave irradiation on the expression of heat shock protein genes in the maize weevil (Coleoptera: Curculionidae). *Eur J Entomol* 113:135–142. <https://doi.org/10.14411/eje.2016.017>
- Wang LJ, Zhou LJ, Zhu ZH, Ma WH, Lei CL (2014) Differential temporal expression profiles of heat shock protein genes in *Drosophila melanogaster* (Diptera: Drosophilidae) under ultraviolet A radiation stress. *Environ Entomol* 43:1427–1434. <https://doi.org/10.1603/EN13240>
- 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>
- Wu SJ, Liu FH, Hu SM, Wang C (2001) Different combinations of the heat-shock cognate protein 70 (hsc70) C-terminal functional groups are utilized to interact with distinct tetratricopeptide repeat-containing proteins. *Biochem J* 359(2):419–426. <https://doi.org/10.1042/0264-6021:3590419>
- Yang XQ, Zhang YL, Wang XQ, Dong H, Gao P, Jia LY (2016) Characterization of multiple heat-shock protein transcripts from *Cydia pomonella*: their response to extreme temperature and insecticide exposure. *J Agric Food Chem* 64:4288–4298. <https://doi.org/10.1021/acs.jafc.6b01914>
- Yoshimi T, Minowa K, Karouna-Renier NK, Watanabe C, Sugaya Y, Miura T (2002) Activation of a stress-induced gene by insecticides in the midge, *Chironomus yoshimatsui*. *J Biochem Mol Toxicol* 16:10–17. <https://doi.org/10.1002/jbt.10018>
- Zhang Q, Denlinger DL (2010) Molecular characterization of heat shock protein 90, 70 and 70 cognate cDNAs and their expression patterns during thermal stress and pupal diapause in the corn earworm. *J Insect Physiol* 56:138–150. <https://doi.org/10.1016/j.jinsphys.2009.09.013>
- Zhao L, Jones WA (2012) Expression of heat shock protein genes in insect stress responses. *Invertebr Surviv J* 9:93–101. <https://doi.org/10.1155/2012/484919>
- Zhou GH, Wen JJ, Cai DJ, Li P, Xu DL, Zhang SG (2008) Southern rice black-streaked dwarf virus: a new proposed *Fijivirus* species in the family *Reoviridae*. *Chin Sci Bull* 53:3677–3685. <https://doi.org/10.1007/s11434-008-0467-2>
- Zhou C, Liu L, Yang H et al (2017) Sublethal effects of imidacloprid on the development, reproduction, and susceptibility of the white-backed planthopper, *Sogatella furcifera* (Hemiptera: Delphacidae). *J Asia Pac Entomol* 20:996–1000. <https://doi.org/10.1016/j.aspen.2017.07.002>
- Zhou C, Yang H, Wang Z, Long GY, Jin DC (2018) Comparative transcriptome analysis of *Sogatella furcifera* (Horváth) exposed to different insecticides. *Sci Rep* 8:8773. <https://doi.org/10.1038/s41598-018-27062-4>
- Zhou C, Yang H, Wang Z, Long GY, Jin DC (2019) Protective and detoxifying enzyme activity and ABCG subfamily gene expression in *Sogatella furcifera* under insecticide stress. *Front Physiol* 9:1890. <https://doi.org/10.3389/fphys.2018.01890>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.