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Identification and expression analysis of four heat shock protein genes associated with thermal stress in rice weevil, *Sitophilus oryzae*

Fei Hu[a,1], Kan Ye[a,1], Xiao-Fang Tu[a], Yu-Jie Lu[b], Kiran Thakur[a], Zhao-Jun Wei[a,*]

[a] School of Food Science and Engineering, Hefei University of Technology, Hefei 230009, People's Republic of China
[b] College of Food Science and Technology, Henan University of Technology, Zhengzhou 450051, People's Republic of China

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ABSTRACT

In order to explore the function of heat shock proteins during thermal stress in rice weevil, *Sitophilus oryzae*, four heat shock protein genes were cloned and characterized. These heat shock protein genes (*hsps*) were named as *Sohsp70–1*, *Sohsp70–2*, *Sohsc70*, and *Sohsp90*, respectively. These *hsps* showed high sequence conservation with the maximum identity with *hsps* of *Tribolium castaneum* and other insects. All the four genes showed the highest mRNA expression in pupal stage and the lowest levels in larval stage. The induced expression of the two *Sohsp70s* (*Sohsp70–1* and *Sohsp70–2*) were reached to the highest levels (15.59-fold and 12.66-fold) after 2 h of incubation at 37 °C, respectively. Expression of *Sohsp90* not only was significantly elevated by heat stress but also by cold stress. Whereas, expression level of *Sohsc70* was not induced either by heat or cold stress. Furthermore, for rapid heat hardening, the expression levels of *Sohsp70–1*, *Sohsp70–2*, *Sohsc70* and *Sohsp90* were observed as 2.57, 2.53, 3.33 and 2.33-fold higher than control, respectively; for rapid cold hardening, the expression levels of *Sohsp70–1*, *Sohsp70–2*, *Sohsc70* and *Sohsp90* were reported as 2.27, 3.02, 3.37 and 2.23-fold higher than control, respectively. Hence, our results revealed that the four *Sohsps* were associated with temperature adaption under rapid heat or cold hardening.

Introduction

Postharvest losses caused by insects, mites, microbes and rodents during grains storage have been reported approximately 10–30% in developing countries (Yadav et al., 2014). It is estimated that the overall damage caused by the pests is equivalent to 5% every year (Zhou and Wang, 2016). The rice weevil, *Sitophilus oryzae*, a Coleoptera beetle of the Curculionidae family, is known for widespread damage; henceforth quality losses and cut down vendibility during storage (Jian et al., 2012).

Until now, phosphine fumigation has been commonly used as an insect control measure for lots of stored products all over the world due to its effectiveness and simple low-cost operation (Rajendran and Sriranjini, 2008). However, this traditional chemical fumigation has led to several adverse effects on human health and environment. Therefore, alternative management methods have been investigated and developed to replace the presented chemical fumigation practice. Previous studies have investigated the alternative physical methods replacing the fumigations such as ionizing irradiation (Carocho et al., 2014), controlled atmosphere (Caleb et al., 2012), cold storage (Aluja et al., 2010), and heating exposure (Alfaifi et al., 2014; Jian et al., 2015; Wang et al., 2013).

In recent years, heat treatments (hot air, hot water, and radio frequency heating) have attracted huge attention and they have been widely studied in laboratories to disinfest postharvest products due to their environmental friendly approach without any use of chemical residues besides easy application and fungicidal effects (Zhou and Wang, 2016). Moreover, heat treatment has been widely studied as an efficient and safe technique (Zhao et al., 2007). Previous studies showed that exposure to 50 °C for about 2 h caused 100% adult mortality of *Tribolium castaneum*, *Rhizopertha dominica* and *S. oryzae* (Arthur, 2006; Tilley et al., 2007). Using the heat block system, the thermal death kinetics of adult *S. oryzae* have indicated that the required holding times for achieving 100% mortality were 130, 50, 12, and 4 min at 44, 46, 48, and 50 °C, respectively (Yan et al., 2014). When radio frequency heating reached up to 50 °C and maintained at 50 °C hot air for 5 min, the mortality levels of adult *S. oryzae* were approximately 99% (Zhou and Wang, 2016). It was reported that after adult *S. oryzae* was exposed to 42–48 °C, the lethal time for 99% mortality was 85 min in 50 g wheat grains (Golić et al., 2016).

* Corresponding author.
E-mail addresses: hufei@hfut.edu.cn (F. Hu), kumarikiran@hfut.edu.cn (K. Thakur), zjwei@hfut.edu.cn (Z.-J. Wei).
[1] Co-first author.

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Among the well recognized molecular responses to thermal extremes in insects, those involving heat shock proteins (*hsps*), are considered to play key roles in environmental stress tolerance and in thermal adaptation (King and MacRae, 2015). In insects, four major families of *hsps* have been recognized on the basis of the molecular mass, amino acid sequences and function, including the small heat shock proteins (shsps), hsp60, hsp70, and hsp90. The *hsp70* family is the largest group of *hsps*, and can be divided into two sub-groups: *hsp70* and heat shock protein cognates 70 (*hsc70*), based on their expression patterns in response to various stimuli (Cheng et al., 2016; Shim and Lee, 2015; Sun et al., 2016). In recent years, an increasing number of studies have indicated that *hsps* expression are induced by various stresses, such as heat, cold, anoxia, desiccation, starvation, heavy metal and insecticides (King and MacRae, 2015; Koo et al., 2015; Lu et al., 2016; Sun et al., 2016; Tang et al., 2015; Tungjitwitayakul et al., 2016; Yang et al., 2016).

However, the knowledge about molecular mechanisms regulated by *hsps* and how these regulations culminate into increased biological responses to *S. oryzae*, is still at its infancy. In the present work, the key purpose was to gain an insight into underlying molecular mechanism of spatiotemporal, responses of rice weevil to thermal stress. Therefore, firstly, we identified the four full-length complementary DNA (cDNA) of *S. oryzae hsp* genes through reverse transcription polymerase chain reaction (RT-PCR), and then after investigated the transcriptional responses of *hsps* in *S. oryzae* responding to different temperatures ranges from lower to higher (−5 °C to 40 °C) by real time quantitative PCR (qPCR).

Materials and methods

Insects

The rice weevil, *S. oryzae* was originally collected from Zhengzhou city, Henan Province, China in the year 2013. Briefly, the insects were reared on whole wheat with 13% grain moisture content in dark photoperiod at 28 °C and 55% relative humidity (RH) in the laboratory. The life cycle of *S.oryzae* at 28 °C was as follows: embryonic stage of 3 day; larval stage of approximately 22 days; pupal stage of 6 days; and adult stage of approximately 6 months. Due to the small size, eggs were difficult to collect inside the grains heap. Hence, 21-days-old larvae, 3-days-old pupae, and 7-days-old adults were collected in the experiments.

Temperature treatment

To induce the thermal shock, 7-d-old adult insects were placed in 2.0 mL microcentrifuge tubes and submerged into a water bath at increasing temperature range of 31, 34, 37, and 40 °C for 2 h. Similarly, tubes were kept at descending temperature range of 10, 5, 0, and − 5 °C for 2 h using a temperature incubator (Sangon Biotech, Shanghai, China). Meanwhile, the insects were exposed to 37 °C or 0 °C for 0, 1, 2, 3, or 4 h to consider the effect of different exposure times. The rice weevils were allowed to recover at 28 °C for 1 h followed by freeze drying in liquid nitrogen and storage at −70 °C. The control populations were maintained at 28 °C and all the treatments were conducted in triplicates for significant statistical analysis.

Rapid heat and cold hardening (RHH and RCH)

For this, 7-d-old adult insects were hardened at sub lethal temperatures [31 °C for rapid heat hardening (RHH) and 5 °C for rapid cold hardening (RCH)] for 2 h followed by exposure to 37 °C or 0 °C for 2 h, respectively. The rice weevils were allowed to recover at 28 °C for 1 h and then frozen in liquid nitrogen and stored at −70 °C. The rice weevils exposed to 37 °C or 0 °C with no hardening period were included as controls. All the treatments were conducted in triplicates for significant statistical analysis.

Table 1
The list of primers used in this study.

Gene	Primer name	Primer sequences (5′ → 3′)	Length (bp)
RT-PCR			
Sohsp70–1	F	ATGGTTAAAGCTCCAGCAATTG	1947
	R	TTAATCTACTTCTTCTATCG	
Sohsp70–2	F	GATCAAAATGGTAAAGACAC	1902
	R	CCATCTTAGTCAACTTCCTC	
Sohsc70	F	ATGGCAAAAGCACCAGCAG	1953
	R	TTAATCGACCTCTTCAATGG	
Sohsp90	F	ATGCCTGAAGAAAGCCAAG	2145
	R	TTAATCGACTTCTTCCATTCG	
qPCR			
Sohsp70–1	F	GCTGGAGATACGCATTTGGG	133
	R	CTGCAGTTCTCAATCGCCTC	
Sohsp70–2	F	ACCTTCACCACATACGCAGA	167
	R	GCATCCAGGTCGAACGTTAC	
Sohsc70	F	CATCTTCCACTCAAGCCAGC	180
	R	CGATGTCATGTACTGCGGAC	
Sohsp90	F	CGAAATCAACCCCGACCATC	168
	R	CGGTAAATCCTGGAAGCGTG	
β-Actin	F	TGCTGACCGAATCCAGAAGG	177
	R	GCCAGGTCCCGATTCATCAT	

Identifying, cloning, and sequencing of hsp genes

Four *hsps* gene fragments from *S. oryzae* were identified by *in silico* analysis of annotated unigenes from the transcriptome data of the rice weevil (http://www.ncbi.nlm.nih.gov/sra/SRX2147201) by imputing keywords such as *hsp* or heat shock protein. Total RNA was extracted from 7-d-old adults using TRIzol reagent (Takara, Dalian, China). For fragments with complete open reading frames (ORFs), full-length sequence accuracy of each *hsp* cDNA was verified using the first-strand cDNA template with confirmation primers (Table 1). The PCR products were cloned in pGEM-T Easy vector (Promega, Madison, WI, USA). DNA sequencing was conducted using an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA). Complete sequences were analyzed by locating similarities between the cloned sequences and existing sequences taken from NCBI database using BLASTX.

Bioinformatics analysis

Nucleotide and amino acid sequence similarities were evaluated using the BLAST programs available at the NCBI website (https://www.blast.ncbi.nlm.nih.gov/Blast). ORFs were identified by ORF finder (http://www.ncbi.nlm.nih.gov/orffinder/). The online tool ScanProsite was used to identify the features of *hsps* families (http://www.expasy.org). The phylogenetic tree was constructed by MEGA 5.0 software using the Neighbor-Joining method (Tamura et al., 2011). The automated protein structure homology modeling sever, Swiss Model (http://www.expasy.org/swissmod/), was used to generate the three-dimensional models of *Sohsp70–1*, *Sohsp70–2*, *Sohsc70* and *Sohsp90* based on the PDB structure 4j8f and 5fw1, respectively.

Real time quantitative PCR

Real time quantitative PCR (qPCR) was used to determine the expression patterns of four *S. oryzae hsp* genes in response to various thermal stresses. Total RNA of all samples at different developmental stages and after treatments by heat or cold was extracted using TRIzol reagent (Takara, Dalian, China). The genomic DNA was removed followed by cDNA synthesis using a PrimeScript RT reagent kit with the gDNA Eraser (Takara, Dalian, China). The qPCR was performed using SYBR Premix Ex Taq II (TliRNaseH Plus) (Takara, Dalian, China) on a Bio-Rad MyIQ2 Real Time PCR system (Bio-Rad, Hercules, CA, USA).