Cloning of the heat shock protein 90 and 70 genes from the beet armyworm, *Spodoptera exigua*, and expression characteristics in relation to thermal stress and development

Xingfu Jiang · Huifang Zhai · Ling Wang · Lizhi Luo · Thomas W. Sappington · Lei Zhang

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Abstract Two full-length cDNAs of heat shock protein (HSP) genes (*Se-hsp90* and *Se-hsp70*) were cloned from the beet armyworm, *Spodoptera exigua*, and their expression was investigated in relation to cold shock, heat shock, and development. The open reading frames of *Se-hsp90* and *Se-hsp70* are 2,154 and 2,004 bp in length, encoding polypeptides of 717 and 667 amino acids with a molecular mass of 82.6 and 72.5 kDa, respectively. Both genes showed high similarity to their counterparts in other species. Transcriptional expression profiles revealed that both genes were significantly up-regulated under thermal stress. However, the temperature at which expression level became significantly higher than that of controls varied between genes. Intensity of response to temperature was more intense for *Se-hsp70* than for *Se-hsp90*, regardless of temperature or developmental stage. However, intensities of response to temperature of either *Se-hsp90* or *Se-hsp70* varied with developmental stage. The basal expression of both genes was highest in young larvae and decreased with age. Translational expression of *Se-Hsp70* was observed by using Western blot, the expression profiles of *Se-Hsp70* protein were in high agreement with those of *Se-hsp70* RNA under heat or cold stress in larvae and pupae. However, it does not completely accord with that of *Se-hsp70* RNA expression during development without ther-

mal stress. These results indicated that, in addition to heat shock responses, both *Se-hsp90* and *Se-hsp70* might be involved in development.

Keywords *Spodoptera exigua* · *Se-Hsp90* · *Se-Hsp70* · Expression · Thermal stress · Development

Introduction

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), is an important polyphagous insect pest on vegetables, cotton, wheat, maize, and ornamental plants (Burris et al. 1994; Suenaga and Tanaka 1997; Jiang and Luo 1998a, 1999). It is also a worldwide pest that occurs in tropical, subtropical, and temperate regions at latitudes between 35° to 40°S and 40° to 57°N (Zhang and Zhao 1996). Originating from South Asia (Gill 1987), it has spread to Europe, Africa, Australia, America and other parts of Asia (Jiang and Luo 1998a; Adamczyk et al. 2003). In South China, including Guangdong, Shenzhen, and Fujian provinces, and Taiwan, the species is adapted to high temperature, occurring throughout the year (Chu and Wu 1992; Jiang et al. 2001). The infesting population usually peaks when local temperatures are highest (Jiang and Luo 1999). In the Yangtze River region of China, severe damage occurs when the summer and autumn temperatures are high, showing a significant positive correlation to temperature (Jiang and Luo 1999).

In addition to heat tolerance, *S. exigua* also exhibits cold resistance, overwintering as larvae and pupae in temperate zones without entering diapause (Fye and Carranza 1973). The pupae have an average supercooling point of approximately −17°C with a low of −21°C in some individuals (Jiang et al. 2001). In southern Europe, southern North

X. Jiang (✉) · H. Zhai · L. Wang · L. Luo · L. Zhang
State Key Laboratory for Biology of Plant Diseases and Insect Pests,
Institute of Plant Protection,
Chinese Academy of Agricultural Sciences,
Beijing 100193, China
e-mail: xjiang@ippcaas.cn

T. W. Sappington
USDA-ARS Corn Insects & Crop Genetics Research Unit
Genetics Laboratory,
Ames, IA 50011, USA

America (Fye and Carranza 1973), Central Asia (Kurdov 1986), and East Asia (Kimura 1991), overwintering pupae were observed in the field. In China, the infesting population reaches northward to Liaoning province (123.29°E, 41.80°N) in Northeast China (Li 2006). In the Yangtse River region and in northern China, overwintering larvae and pupae also have been observed in the field (Zhou and Xu 1993; Yin et al. 1994; Lu and Xie 1995).

Notably, this species undertakes a long distance migration between or within tropical, subtropical and temperate regions (French 1968; Mikkola 1970; Yathom 1971; Oku and Kobayashi 1978; Mitchell 1979; Kimura 1991; Jiang and Luo 1998b; Feng et al. 2003; Niu et al. 2006), which significantly increases the geographic range over which infestations occur. Life history strategies of *S. exigua*, such as overwintering (Jiang et al. 2001), migration, and reproduction under various temperature, humidity, density, and food conditions, have been well documented in our previous studies, showing substantial environmental tolerance and ecological plasticity in this species (Jiang and Luo 1998b; Jiang et al. 1999, 2000, 2002, 2010; Wang et al. 2008). Furthermore, *S. exigua* is also known for its rapid development of resistance to many chemical pesticides and its lack of susceptibility to transgenic Bt crops (Wang et al. 2002; Lin et al. 2007; Wu et al. 2008; Jia et al. 2009; Lan and Zhao 2010). Therefore, it can be presumed that *S. exigua* has a high potential to tolerate or acquire tolerance to various environmental stresses. However, the biochemical and molecular mechanisms of such tolerance remain unknown.

The several families of heat shock proteins (HSPs) have been widely studied in a wide range of organisms (Feder and Hofmann 1999). The HSP genes *hsp70* and *hsp90*, which are found in most organisms, are highly conserved in all eukaryotes and prokaryotes so far studied (Huang et al. 2008). Up-regulation of *hsp70* and *hsp90* mRNA levels in response to cold and heat shock, density, starvation, and diapause has been reported in several insects (Schlesinger 1990; Goto et al. 1998; Goto and Kimura 1998; Rinehart et al. 2000, 2007; Yocum 2001; Hayward et al. 2005; Sinclair et al. 2007; Huang et al. 2007; Huang and Kang 2007). However, there is no information about the stress-induced HSP expression in *S. exigua*, which limits our understanding of the mechanisms underlying this species' ecological adaptability and stress tolerance. In addition, HSPs may be involved in the developmental processes of some insects (Mahroof et al. 2005a, b; Sonoda et al. 2006; Huang et al. 2009). These studies have provided direct evidence of the roles of HSPs in cellular activity and development, and have elucidated important biological functions.

This study examines basal gene expression and thermal responses among different developmental stages in larvae

and pupae of *S. exigua*, using cloning, sequencing and expression profiling of *Se-hsp90* and *Se-hsp70*. Furthermore, we identify *Se-Hsp70* expression at the protein level under heat or cold stress in larvae and pupae, and basal expression during development. We explore the functions of these genes and protein in the context of thermotolerance and development, and provide information needed to explore the mechanism of environmental tolerance and ecological adaption in *S. exigua*.

Materials and methods

Insects

Experimental *S. exigua* were from a laboratory colony started with insects originally collected on onion in Beijing, China in 2006. Larvae were reared on artificial diet under constant temperature of $26 \pm 1^\circ\text{C}$ and a photoperiod of L/D 12:12, as described by Jiang et al. (1999).

Heat and cold shock during development

Based on previous studies of thermotolerance in this species (Ma et al. 1999; Jiang et al. 2001), developing larvae from the second to fifth instar were selected for heat shock treatment, and second to fourth instar larvae and 2-day-old pupae were exposed to cold shock. For each treatment, five individuals were placed into a cryogenic tube, and shocked at high temperatures of 37, 39, 41, 43, and 45°C , respectively, for 1 h, then allowed to recover at 26°C for 1 h. Similarly, target samples were shocked at low temperatures of 5 or 0°C for 3 h and -10°C for 2 h, respectively, then transferred to 26°C for 1 h recovery. Corresponding untreated larvae and pupae were used as controls. Each treatment was replicated three times. For detecting developmental regulation of *Se-hsp90* and *Se-hsp70* genes, each treatment included five individuals of each larval instar, and 2-, 4-, and 6-day-old pupae reared at 26°C , replicated three times. All samples were frozen quickly in liquid nitrogen and stored at -70°C . Temperature control was achieved by environmental chambers (Sanyo, Tokyo, Japan).

Cloning the full-length cDNAs of *Se-hsp90* and *Se-hsp70*

Reverse transcription-polymerase chain reaction (RT-PCR) and RACE methods were used to clone full-length cDNAs encoding *Se-hsp90* and *Se-hsp70*. Total RNA was isolated with Trizol (Invitrogen) following the manufacturer instructions. The quality and quantity of the total RNA were assessed using a spectrophotometer (NanoDrop 2.5.1). First-strand cDNA synthesis was performed using the

Quantscript RT kit (TransGen Biotech, Beijing, China) following the recommended protocol of the manufacturer. The resulting cDNA was used as a template for PCR with degenerate oligonucleotide primers designed on the basis of known sequences from *hsp70* and *hsp90* of other insects. The sequences of all primers used in this study are summarized in Table 1. Using the degenerate primer pairs *hsp90F1*, *hsp90R1*; *hsp70F1*, *hsp70R1*, *Se-hsp90*, and *Se-hsp70* gene fragments were successfully amplified with Ex-Taq (TAKARA, Dalian, China), under the condition of 30 cycles at 94°C for 30s, 53°C for 30s, and 72°C for 1 min, followed by a final extension at 72°C for 10 min. Purified PCR products were inserted into the pEASY-T3 Cloning Vector (TransGen Biotech, Beijing, China) and subsequently sequenced (Shanghai Sangon, Beijing, China).

To obtain the complete cDNAs of *Se-hsp90* and *Se-hsp70*, respectively, gene-specific primers (*hsp90GSP1*, *hsp90GSP2*; *hsp70GSP1*, *hsp70GSP21*) were designed matching the primers in the 3' and 5' Full RACE kit (TAKARA). Both 3'- and 5'-RACE PCR was carried out under the same conditions described above. The amplified PCR products of 3'-RACE and 5'-RACE were inserted into the pEASY-T3 Cloning Vector (TransGen Biotech, Beijing, China) and sequenced (Shanghai Sangon). To ensure against sequencing errors, specific primer pairs (*hsp90Adj-S* and *hsp90Adj-A* for *Se-hsp90*, and *hsp70Adj-S* and *hsp70Adj-A* for *Se-hsp70*) were used to amplify the predicted gene including the entire open reading frame, and the PCR products were cloned into the Eeasy-T3 vector and sequenced.

Sequence alignment and identity analysis

Full-length cDNAs of *Se-hsp90* and *Se-hsp70* were used to search for homologs in GenBank by BLAST software available at the NCBI website (<http://www.ncbi.nlm.gov/blast/>). The sequence alignment and identity analysis were carried out using the DNAMAN software package, and ORFs were identified with the aid of the software of DNASTAR and DNASIS. Molecular weights of the predicated proteins were calculated by PROTPARAM and PROSITE software.

Real-time fluorescence quantitative PCR

Total RNA was extracted using Trizol (Invitrogen) and quality assessed by spectrophotometry (NanoDrop 2.5.1). Five hundred nanograms of total RNA from each sample was reverse transcribed with PrimeScript™ RT reagent Kit (Perfect Real Time, TAKARA). Each pair of primers was designed based on the highly conserved regions of the cDNAs of *Se-hsp90* and *Se-hsp70* and the β -actin gene from *S. exigua* using primer 5.0 software. Conventional RT-PCRs for *Se-hsp90* and *Se-hsp70* and β -actin genes were carried out using Ex-Taq polymerase (TAKARA) and the corresponding primers (Table 1) using the same amplification conditions described above. The PCR amplification products were analyzed, cloned and sequenced as above. Real-time quantitative PCR for *Se-hsp90*, *Se-hsp70*, and β -actin genes were carried out on an iCycler iQ (Bio-Rad, USA) using SYBR Premix Ex-Taq (TAKARA) under the following conditions: 95°C for 10 s, followed by 40 cycles

Table 1 Primer sequences used in the cDNA cloning and real time quantitative PCR of *S. exigua* (*Se*) heat shock protein (*hsp*) genes

Gene	Primer name	Primer sequence (5'-3')	Use
<i>Se-hsp90</i>	<i>hsp90F1</i>	TGATC(T)GGTCAGTTC(T)GGTGTGG	PCR
	<i>hsp90R1</i>	A(G)TA(G)GGTCCTCACAGTTGTCCAT	
	<i>hsp90GSP1</i>	CTGCT(A)CAGTAT(C)GGTTGGTCTGCC	3'-RACE
	<i>hsp90GSP2</i>	GGT(C)CTTCCTTGATGTG(C)GAGA(C)ACGAT	5'-RACE
	<i>hsp90FQ</i>	CTGGAACCTAAGGCATTATGAGGCC	Real-time quantitative PCR
	<i>hsp90RQ</i>	CTGCTCGTCATTGTGCTTAG	
<i>Se-hsp70</i>	<i>hsp70F1</i>	G(T)ATATGAAGCACTGGCCGTT	PCR
	<i>hsp70R1</i>	GACAGGTTTCAGCTTCTTGCC(G)	
	<i>hsp70GSP1</i>	CCGAGAAGTACAAGGACGAGGA	3'-RACE
	<i>hsp70GSP2</i>	CTTTGAAGGGCCAGTGCTTCATGTC	5'-RACE
	<i>hsp70FQ</i>	TACCACATACTCGTGCCTCG	Real-time quantitative PCR
	<i>hsp70RQ</i>	TTCTTGCTGCGTCTCCGA	
β -actin	<i>Actin-S</i>	TCCAGCCTTCCTTCTTGGGTAT	Real-time quantitative PCR
	<i>Actin-A</i>	CAGGTCCTTACGGATGTCAACG	
<i>Se-hsp90</i>	<i>hsp90Adj-S</i>	GTGAATCAGTCAAATGCCCGAAGA	Adjust for <i>hsp90</i>
	<i>hsp90Adj-A</i>	CTGAGATTTTAATCCTCTTCTCCCA	
<i>Se-hsp70</i>	<i>hsp70Adj-S</i>	AATCTGAATACAAAATGCCAGCCAT	Adjust for <i>hsp70</i>
	<i>hsp70Adj-A</i>	TTTCTATTTAATCAACTTCTTCAA	

at 95°C for 5 s, and 60°C for 30 s. To evaluate the specificity of the SYBR Green real-time RT-PCR, a melting curve temperature was determined using the dissociation curves obtained from the PCR products and the derivatives ($-dF/dT$) of fluorescence values plotted at 0.5°C intervals from 55°C to 94°C.

Western blot analysis

To identify *Se-Hsp70* expression at protein level after thermal stress, the fourth instar larvae and pupae were selected to perform western blot. Total proteins were extracted using Tissue Protein Extraction Reagent kit (Cangwei Biotech. Co., Beijing) according to the manufacturer's manual. Total protein concentrations were determined using the bicinchoninic acid (BCA) method (Tiangen Biotech, Beijing Co., Ltd.). Western blot analysis was performed according to Towbin et al. (1979) with small modification. Protein samples were electrophoresed on 8% SDS-polyacrylamide gel and electroblotted to NC membrane (Protran Dassel, Germany). Transfer was run at 85 mA for 10.5 h, using Tris/glycine buffer. Membranes were blocked with 5% nonfat powdered milk in Tris-buffered saline containing 0.05% Tween 20 (TBS-T) and incubated for 2 h at 37°C. The primary antibody against HSP70 (Monoclonal anti-heat shock protein 70 antibody produced in mouse; Sigma, St. Louis, MO), which is specific for the inducible form of Hsp70 in *Drosophila*, was used at 1:1,000 dilution and incubated for 2 h at 37°C. The membrane was washed three times in PBS and once in TBS-T, 10 min each, and then incubated with HRP conjugated goat anti-mouse IgG secondary antibody (Boisynthesis Biotech. Co., Ltd. Beijing) diluted 1:750 in blocking buffer for 1 h at 37°C. The membrane was washed three times with TBS-T and once in PBS, 10 min each, and then developed in DAB substrate buffer for 10 min. For each experiment, samples containing an equal amount of total protein were run on the same gel. Densitometric analysis of the immunoblots was performed using a desktop scanner (Hanwang E60) and Image J free software (<http://rsb.info.nih.gov/ij/index.html>). Western blotting was replicated three times.

Data collection and statistical analysis

The qPCR standard curves constructed in this study were used to quantify expression data of *Se-hsp90* and *Se-hsp70*. Each gene's cDNA recombinant plasmid concentration was determined by spectrophotometry (NanoDrop 2.5.1), then serially diluted 10-fold. Two technical replicates were used to produce the standard curve, and three replicates for samples. For all SYBR Green quantification real-time RT-PCR assays, a no-template control was included. The

reference gene of β -actin was used as an endogenous control when quantification of target genes was determined. This gene is an appropriate control in this insect during this wide developmental window as determined in our previous studies (Tian et al. 2009; Cheng et al. 2010). The average concentration across replicates was obtained from the standard curves and the relative expression ratio of each target gene was calculated according to protocols established by the manufacturer (ABI Prism 7700 Sequence Detection System). The mathematical models used for relative quantification of target genes were the same as described by Rasmussen (2001) and Pfaffl (2001). All the numerical data obtained from the quantitative tests of *Se-hsp90*, *Se-hsp70* and *Se-Hsp70* protein were presented as mean $\pm$ SEM. Statistical analyses were conducted using SPSS V. 16.0 software, applying one-way ANOVA and Tukey's HSD test.

Results

Cloning and characterization of *Se-hsp90*

The complete nucleotide and deduced amino acid sequences of *Se-hsp90* are presented in Fig. 1 (GenBank accession no. FJ862050). There was a 5'-untranslated region of 163 bp, followed by an initiation codon (ATG). The cDNA contained 2,154 bp of open reading frame (ORF), which codes for a putative protein of 717 amino acids with a calculated molecular weight of 82.6 kDa. The termination codon (TAA) occurred at nucleotide position 2,315. The polyadenylation signal (AATAAA) started 111 nucleotides downstream from the end of the termination codon. The poly (A) tail occurred at 20 nucleotides downstream from the end of the polyadenylation signal. All five highly conserved segments defining the *hsp90* family signature in eukaryotes, NKEIFLRELISN(S/A)SDALDKIR, LGTIA(K/R)SGT, IGQFGVGFYS(A/C)(Y/F)LVA(E/D), IKLYVRRVFI, and GVVDS(E/D)DLPLN(I/V)SRE (Gupta 1995), are well conserved in *S. exigua* (Fig. 1). The terminal amino acid sequence EEVD, constituting the core of the HSP90 interaction surface for the tetratricopeptide repeats of HSP90 co-chaperones (Ramsey et al. 2000; Scheufler et al. 2000), and shared with the HSP70 gene family, is strictly conserved.

The deduced amino acid sequence of *Se-hsp90* displayed a high degree of similarity with other insects. The highest amino acid identity of 99% and 98% were observed with the *hsp90* from the noctuids *Spodoptera frugiperda* (GenBank accession no. AF254880) and *Mamestra brassicae* (GenBank accession no. AB251894), respectively; 84% and 83% from *Liriomyza sativae* (GenBank accession no. AY851368) and *Drosophila melanogaster* (GenBank acces-

	CATTTAATTCAAGAAAGCAAAGTGAGAGATACGCTGCGATTCCACGCGATCTTACAACACGTGTATTAACCC
1	GCGTAGTCAAGAGATTTTAGCAAGTGTCTGTGCTTACTGTATTCTGTTACTTCGAAGAATTTAAATTAATTAAGTGAATCAGTCAAA
1	ATGCCGAAGAAATGCAGACCGATGTTGCCGAGGTGGAGACCTTCGCCTTCCAGGCGGAGATCGCCAGCTTATGTCCTGATCATCAAC
91	M P E E M Q T D V A E V E T F A F Q A E I A Q L M S L I I N
31	A CATTCTACTCCAACAAAGAGATCTTCCTTCGTGAGTTAATCTCCAACCTTTCAGACGCCTTGGACAAGATCCGTTATGAGTCACTACC
181	T F Y S <u>N K E I F L R E L I S N S S D A L D K I R Y E S L T</u>
61	G ACCCATCGAAGCTGGACAGCGCAAGGACCTGTACATCAAGATCATTCCCAACAAGAGCGAAGGTACACTCACTATTATTGATACCGGT
271	D P S K L D S G K D L Y I K I I P N K S E G T L T I I D T G
91	A TTGGTATGACCAAGGCTGACCTCGTCAACAACCTTGGTACCATCGCCAAGTCTGGAATAAGGCATTTCATGGAGGCCCTGCAGGCAGGC
361	I G M T K A D L V N N <u>L G T I A K S G T</u> K A F M E A L Q A G
121	G CTGACATTAGCATTTGGTCAGTTCGGTGTGGTTCCTACTCATCTCTGCTGCTGACCGCGTACTGTTCACTCAAGCACAAT
451	A D I S M <u>I G Q F G V G F Y Y S C Y L V A D</u> R V T V H S K H N
151	G ACGACGAGCAGTACCGGTGGGAGTCGTCGGCGGAGGCTCGTTACAGTCCGCCCTGACCATGACGAGCCCTCGGACGCGGTACAAAG
541	D D E Q Y R W E S S A G G S F T V R P D H D E P L G R G T K
181	A TCGTTCTCCACATCAAGGAAGACCTCACTGAGTACCTTGAAGAGCACAAGATTAAGGAAATCGTCAAGAAGCACTCGCAGTTCATCGGC
631	I V L H I K E D L T E Y L E E H K I K E I V K K H S Q F I G
211	C CCATCAAACCTGATGTACGTTGAGAAGAACGTGAGAAGGAGCTCTCTGATGATGAGCCGAGGAGGAGAAGAAAGAGGATGAAAAGGAG
721	Y P I K L M V E K E R E K E L S D D E A E E E K K E D E K E
241	G ATGACAAGCCTAAGATTGAGGATGTGGGAGAGGATGACGAAGAGGACAAGAAAGATAAGAAGAAGAAGACCATCAAGGAGAAATAC
811	D D K P K I E D V G E D D E E D K K D K K K K K T I K E K Y
271	A CAGAGGACGAGGAACCTCAACAAGACCAAGCCATCTGGACCCGTACGCCGACGATATCACACAGGAGGAGTATGGTGACTTCTACAAA
901	T E D E E L N K T K P I W T R N A D D I T Q E E Y G D F Y K
301	T CCCTACCAATGACTGGGAGGACACCTTGCTGTCAAACACTTCTCAGTTGAAGGTCAAGTGGAGTTCAGAGCCTTGTGTCTGTGCC
991	S L T N D W E D H L A V K H F S V E G Q L E F R A L L F V P
331	C GCGGTGCTCCCTTCGACTTGTGTTGAGAACAAGAAGCGCAAGAACAACATCAAACCTGTACGTCGCCAGAGTATTCATCATGGACAACCTGT
1081	R R A P F D L F E N K K R K N N <u>I K L Y V R R V F I M D N C</u>
361	G AGGACCTTATCTGAATACCTGAACCTTATCAAGGGTGTGTAGACAGTGAGGACCTGCCCTCAACATCTCTCGTGAGATGCTTCAA
1171	E D L I P E I K I E D V G E D D E E D K K D K K K K K T I K E K Y
1261	C AGAACAAGATCCTTAAAGTAATCAGAAAGAACTTGGTTAAGAAATGCTTGGAACTCTTCGAAGAGTTGGCTGAAGACAAAGAGAACTAC
421	Q N K I L K V I R K N L V K K C L E L F E E L A E D K E N Y
1351	A AGAAGTATTACGAACAGTTACGAAGAACCTGAAGCTTGGTATCCATGAGGACTCTCAGAACAGGTCAAACCTGGCTGACTTGCTCCGC
451	K K Y Y E Q F S K N L K L G I H E D S Q N R S K L A D L L R
1441	T ACCACACATCTGCCTCTGGTGATGAGGCATGCTCCCTAAAGAGTATGTCTCAGTATGAAGGAGAACCAGAACACATCTACTACATC
481	Y H T S A S G D E A C S L K E Y V S R M K E N Q K H I Y Y I
1531	A CTGGTGAGAACCCTGACCAAGTTGCCAATCATCATTGTAGAGCGAGTCAAGAAGCGTGGTTATGAAGTTGTATACATGACTGAGCCT
511	T G E N R D Q V A N S S F V E R V K K R G Y E V Y M T E P
1621	A TTGATGAGTATGTATACAGCAGATGAAAGAGTATGAGTGAAGCTTGTCTCTGTACAAAGGAGGCTTGGAACTCCCTGAAGAT
541	I D E Y V V Q Q M K E Y D G K T L V S V T K E G L E L P E D
1711	G AAGAAGAAAAGAAGCGTGAGGAGGACAAGGTGAAGTTCGAGGGTCTCTGCAAGGTCATGAAGAATCCTTGACAACAAAGTCGAG
571	E E E K K K R E E D K V K F E G L C K V M K N I L D N K V E
1801	A AGGTTGTGTATCTAACCGTCTGTGTGAGTCGCCATGCTGCATTGTCACTGCTCAGTATGGTTGGTCTGCCAACATGGAACGTATCATG
601	K V V V S N R L V E S P C C I V T A Q Y G W S A N M E R I M
1891	A AGGCCAGGCTCTCCGTGACACTGCCACCATGGGTTACATGGCAGCAAGAAACACCTTGAATTAACCTGACCATTCATTGTTGAG
631	K A Q A L R D T A T M G Y M A A K K H L E I N P D H S I V E
1981	A CCCTGAGGCAGAGGAGGCTGACAAGAAGGCTGTCAAGGACCTGTCATCCTTTTGTACGAGACTGCCCTGCTGTCTTCT
661	T L R Q K A E A D K N D K A V K D L V I L L Y E T A L L S S
2071	G GCTTACCCTAGACGAGCCCCAAGTGCACGCCTCTCGCATCTACCGCATGATCAAGCTTGGTCTTGGCATCGACGAGGACGAGCCATC
691	G F T L D E P Q V H A S R I Y R M I K L G L G I D E D E P I
	C AGGTTGAGGAGTCGAGCGCGGCGACGTGCCCCGCTAGAGGGCAGCTGACGACGCTGACGTATGGAGGAAGTGATTAA 3'
	Q V E E S S A G D V P P L E G D A D D A S R M E E V D *
	AATCTCAGCTTTTAGCCATGTTGTAATGGTGTATGTGATTGTATCGCAAGTTATTTGTGCACTGGTGTACAGCCAAAGACTGATT
	AGTTCAAATTCATTATTTCAAAATGTTGACCTTAACAAACCAA

Fig. 1 Nucleotide and deduced amino acid sequences of *S. exigua* *hsp90* cDNA. The asterisk indicates the translational termination codon (TAA). The putative polyadenylation signal (AATAAA) is **bold and double-underlined**. Five highly conserved regions representing

signature amino acid sequences for HSP90 homologs (Gupa, 1995) are **bold and underlined**. The characteristic HSP cytoplasmic carboxyl terminal region (EEVD) is indicated

sion no. NM079175), respectively. It even shared an 81% and 80% similarity with *Homo sapiens* (GenBank accession

no. NM-005348) and *Rattus norvegicus* (GenBank accession no. NM-175761), respectively.

Cloning and characterization of *Se-hsp70*

The complete nucleotide and amino acid sequences of *Se-hsp70* are presented in Fig. 2 (GenBank accession no. FJ862049). It contains a 155-bp 5'-untranslated region followed by an initiation codon (ATG). The cDNA contained a 2,004-bp ORF, which codes for a putative protein of 667 amino acids with a calculated molecular weight of 72.5 kDa. The termination codon (TAA) occurred at nucleotide 2,156. The polyadenylation signal (AATAAA) was present 147 nucleotides downstream from the end of the termination codon. Three highly conserved HSP70 gene family signatures were also observed in *Se-hsp70*, including IDLGTTYS, IFDLGGGTFDVSIL, and VLVGGSTRIPKVQ, at amino acid positions 6–13, 194–207, and 333–345, respectively (Fig. 2). A putative ATP-GTP binding site, AEAYLGTT (Saraste et al. 1990), is located at amino acid positions 128–135 (Fig. 2). The putative bipartite nuclear localization signal involved in the selective translocation into the nucleus (Knowlton and Salfity 1996) (KK and RRLRT at positions 248–249 and 259–263, respectively) is also highly conserved. The cytoplasmic HSP70 carboxyl terminal region, EEVD, is located at positions 664–667 (Fig. 2).

The deduced amino acid sequence of *Se-hsp70* also displayed a high degree of similarity with other insects, sharing 94% and 91% identity with *hsp70* from *M. brassicae* (GenBank accession no. AB251894) and *Sesamia non-agrioide* (GenBank accession no. EU430480.1), respectively, and 79% and 77% with that from *D. melanogaster* (GenBank accession no. NM079175) and *Aedes aegypti* (GenBank accession no. FJ177309.1), respectively.

Expression of *Se-hsp90* RNA in relation to thermal stress during development

The relative mRNA expression levels of *Se-hsp90* during development induced by low and high temperatures were quantified by real-time quantitative PCR (Fig. 3). *Se-hsp90* expression was not significantly induced by 3 h cold shock at 5 or 0°C, or by 1 h heat shock at 37 or 39°C at any developmental stage tested. However, it was significantly up-regulated at temperatures of −10°C or ≥41°C ($P < 0.05$), except that *Se-hsp90* expression levels induced by −10°C in the fourth instar larvae or by 41°C in the third instar larvae were not significantly different from the controls ($P > 0.05$). *Se-hsp90* expression level in the third instar larvae induced by 45°C decreased significantly compared to that at 43°C ($P < 0.05$). Therefore, the onset temperatures at which the expression level was significantly higher than that of controls of *Se-hsp90* during cold and heat shock in most developmental stages were −10 and 41°C, respectively. The intensities of the temperature response of *Se-hsp90* varied among developmental stages. Higher increases of 5.9- and 9.3-fold appeared in

the second instar larvae and pupae at −10°C under cold shock, respectively, while the greatest increases were observed in second and third instars under heat shock, with a maximum of 6.9-fold in second instars (Fig. 3).

Expression of *Se-hsp70* RNA in relation to thermal stress during development

No significant increase in relative mRNA expression levels of *Se-hsp70* were observed when larvae and pupae were stressed by 5°C or 0°C, or when larvae were stressed from 37 to 41°C (Fig. 4, $P > 0.05$). However, it was significantly up-regulated at temperatures of −10 or ≥43°C, respectively ($P < 0.05$). Therefore, the onset temperatures of *Se-hsp70* up-regulation during cold and heat shock in all tested developmental stages were −10 and 43°C, respectively. The intensities of the temperature response of *Se-hsp70* varied among developmental stages, showing similar expression profiles as those of *Se-hsp90* during development. However, the fourth instar showed the greatest up-regulation of *Se-hsp70* by far (Fig. 4).

Comparative mRNA expression profiles of *Se-hsp90* and *Se-hsp70* induced by cold and heat during development indicated three obvious characteristics (Figs. 3 and 4). First, the onset temperature varied between *Se-hsp90* and *Se-hsp70*, depending on development in the case of heat shock, but not cold shock, with 41°C for *Se-hsp90* in most developmental stages and 43°C for *Se-hsp70* in all tested stages. Secondly, the temperature responses of *Se-hsp70* at any given temperature or developmental stage are more intense than those of *Se-hsp90*. The highest increase was 2,733.3-fold in *Se-hsp70*, whereas it was less than 10-fold in *Se-hsp90*. Finally, intensity of temperature response of either *Se-hsp90* or *Se-hsp70* varied among developmental stages, with the greatest occurring in second instars and pupae in the case of cold shock, respectively, and in larvae younger than the fourth instar in the case of heat shock.

Expression profile of *Se-hsp90* and *Se-hsp70* RNA at different developmental stages

Both *Se-hsp90* and *Se-hsp70* expression decreased with the development of the larva and pupa in the absence of thermal stress (Fig. 5). Expression decreased dramatically with larval age, and the lowest expression level was observed in the fourth instar larvae. Neither *Se-hsp90* nor *Se-hsp70* expression levels changed significantly with aging of the pupa.

Expression of Se-Hsp70 protein in relation to thermal stress and development

A western blot analysis of *Se-Hsp70* protein levels of the fourth instar larvae after cold and heat shock was carried

	TGGAAAAGAATTATTGAAACGCGATACAAAGCAGACGAAGAGAGTTTCGGTGCAAACCGAGAAGT
1	T TGAGTTTCTAGACTCGCAACGCAAGTTGATTCAACGTTATACCACTGTTACTGTTTAGTGTCAAGTGTGAAGAGAATCTGAATACAAA
1	ATGCCAGCCATTGGAATAGATCTGGGTACCATACTCGTGGCTCGGCGTGTGGCAGCACGGCAACGTTGGAGATCATCGCCAACGACCAG
91	M P A I G <u>I D L G T T Y S</u> C V G V W Q H G N V E I I A N D Q
31	GGCAACCGCACCACACCATCTATGTGGCGTTACGCGACCGGAGCGCCTCATCGAGACGCGCAAGAACCAGGTCGCCCTCAACCCC
181	G N R T T P S Y V A F T D T E R L I G D A A K N Q V A L N P
61	AACAACACTGTGTTTCGACGCCAAGCGACTGATCGGAAGGAAATTCGATGACCCCAAGATCCAGGCAGACATGAAGCACTGGCCCTTCAGG
271	N N T V F D A K R L I G R K F D D P K I Q A D M K H W P F R
91	GTGGTCAGCGACTGTGGCAAACCGAAGATCCAAGTGGAGTTCAAGGGTGAAACGAAACGGTTCGCGCCCGAGGAGATCAGCAGCATGGTG
361	V V S D C G K P K I Q V E F K G E T K R F A P E E I S S M V
121	CTGACGAAGATGAAGGAGACGGCGGAAGCTTACCTCGGAACGACAGTACGCGACGCACTGATCACAGTGCCTGGCTACTTCAACGACTCT
451	L T K M K E T A E A Y L G T T V R D A V I T V P A Y F N D S
151	CAGCGCCAGGCCACCAAGGACGCGGGAGCCATCGCCGGCTGAACGTGCTCAGGATCATCAACGAGCCACAGCCGCTGCGCTCGCCTAC
541	Q R Q A T K D A G A I A G L N V L R I I N E P T A A A L A Y
181	GGCCTCGACAAGAACCTCAAGGGCGAGAGGAACGTCCTATTTTCGACCTCGGCGCGGAACGTTCCAGCTGTCGATCTGACCATCGAC
631	G L D K N L K G E R N V L <u>I F D L G G G T F D V S I L T I D</u>
211	GAAGGTTCATTGTTTCAAGTGAGGGCGACGGCGGGCGACACCGACCTCGGAGCGGAGACTTCGACAACAGATTGGTGAACCATCTCGCG
721	E G S L F E V R A T A G D T H L G G E D F D N R L V N H L A
241	GACGAGTTCAAGCGCAAGTACAAGAAGACATGCGCATGAATCTCGCGCACTGCGCCGCTCCGCACAGCCCGGACCGCACTAAGCGC
811	D E F K R K Y K K D M R M N P R A L R R L R T A A E R T K R
271	ACGCTGTCGTCCAGCACCGAAGCCACCATCGAGATCGACGGCTCTACGAGGGAATCGACTTCTACACTCGCGTGTCCCGCGCGCGCTTC
901	T L S S S T E A T I E I D A L Y E G I D F Y T R V S R A R F
301	GAGGAGCTCAACGCTGACCTGTTCCGAGGCACCTCGAGCCGTCGAGAAACGCTCAAGGACGCCAAGATGGACAAGAGCCAGATCCAC
991	E E L N A D L F R G T L E P V E K A L K D A K M D K S Q I H
331	GACGTCGTTCTCGTCGGAGGCTCGACCAATCCCGAAGGTGCAGAGTCTGCTGCAAACTTCTTCTGTGGCAAGAAGCTGAATTTGTCC
1081	D V <u>V L V G G S T R I P K V Q</u> S L L Q N F F C G K K L N L S
361	ATCAACCCGACGAGCGGTTCGGTATGGCGCGGCGTCCAGCGCGCATCTGAGTGGCGAACAAGACTCGAAGATTCAAGCATGTTGTG
1171	I N P D E A V A Y G A A V Q A A I L S G E Q D S K I Q D V L
391	CTGGTGGACGTGGCCCTCTGTCCCTCGGCATCGAAACCGCCGCGCGGTGATGACGAAGATCATCGAGCGCACTGTAAGATCCCGTGC
1261	L V D V A P L S L G I E T A G G V M T K I I E R N C K I P C
421	AAGCAGTCCGACGCTTACACGCTACTCGGACAACACGCCAGCGGTCACGATCCAAGTGTACGAGGGCGAGCGAGCATGACCAAGGAC
1351	K Q S Q T F T T Y S D N Q P A V T I Q V Y E G E R A M T K D
451	AACAACCTGCTGGGCACGTTTCGACTTGACCGCATCCCGCCCGCGCTCGCGGTCTCCCTAAGATCGAGCTACCTTCGACCTGGACGCG
1441	N N L L G T F D L T G I P P A P R G V P K I D V T F D L D A
481	AACGGCATTTCTAACGTGTGCGCCAAAGAGAACAGCACCGGCCGAAGCAAGAACATCGTGATCAAGAACGACAAGGGACGCTGTGCGAG
1531	N G I L N V S A K E N S T G R S K N I V I K N D K G R L S Q
511	GCCGAGATCGAGCGCATGCTCGCGAGGCCGAGAAGTACAAGGACGAGGATGAGAAGCAGAGCGAGCGGTACGCTCTCGGAACGAGCTC
1621	A E I E R M L A E A E K Y K D E D E K Q R Q R V A S R N Q L
541	GAGTCGTACTGTTACCGCTGAAGCAGGCGTGGACGAGCGCGGAGACAAGCTCTCGGAACAGGACAAGAGCAGCGGAGGACGCGTGC
1711	E S Y V F S V K Q A L D E A G D K L S E Q D K S T A R D A C
571	GACGACGCGCTCAAGTGGCTCGACAACAACGCTGGCGGAGCAGGAGGAGTACGAGCACAAGCTGAAGGACGTGCACCGCGTGTGCTCG
1801	D D A L K W L D N N T L A E Q E E Y E H K L K D V Q R V C S
601	CCCGTCATGGCCAAGATGCATGGGGCGGCGCGAAGTGGCGGCGGATGCCCGAGGTATGCCCTGGTGGCATGCCCGGAGGAATGCCT
1891	P V M A K M H G A G A Q G A G G M P G G M P G G M P G G M P
631	GGAGGAATGCCGGCGGAATGCCTGGTGGCATGCCCGGCGGTATGCCAGGAGGAATGCCTGGCGGTACGGAGGAAACCAACAGCGGA
1981	G G M P G G M P G G M P G G M P G G M P G G Y G G N H N S G
661	CCCACCATGAAGAAGTTGATTA 3'
	P T I E E V D *
	ATAGGAAAAATTTGTCAATCTAATTTATGTGATATAAACTATTTTAAGACTGAAATCTAGTGATTAAGTATAGTAATTACTAAAACAA
	ATTCAACAGAGGAAAAATGTGCGTGTGAATGATCTCTAGTCAAATTTGTGTAGAAAAATAAATTAT

Fig. 2 Nucleotide and deduced amino acid sequences of *S. exigua* *hsp70* cDNA. The asterisk indicates the translational termination codon (TAA). The putative polyadenylation signal (AATAAA) is **bold and double-underlined**. Three highly conserved regions representing

signature amino acid sequences for HSP70 homologs are **bold and underlined**. The characteristic HSP cytoplasmic carboxyl terminal region (EEVD) is indicated

Fig. 3 *Se-hsp90* mRNA expression profiles induced by cold (−10 to 5°C) and heat (37–45°C) in second (a), third (b), fourth (c), fifth (d, filled square) instar larvae and pupae (d, empty square) of *S. exigua*. The fifth instar larvae did not survive at 45°C and therefore the mRNA was not detected. The relative level of *Se-hsp90* represents the fold increase above the untreated control (26°C). Data are presented as mean ± SEM. Means with the same letter within cold (lowercase letters) or heat (uppercase letters) treatments are not significantly different at the 5% level by Tukey's HSD

Fig. 4 *Se-hsp70* mRNA expression profiles induced by cold (−10 to 5°C) and heat (37–45°C) in second (a), third (b), fourth (c), fifth (d, filled square) instar larvae and pupae (d, empty square) of *S. exigua*. The fifth instar larvae did not survive at 45°C and therefore the mRNA was not detected. The relative level of *Se-hsp90* represents the fold increase above the untreated control (26°C). Data are presented as mean ± SEM. Means with the same letter within cold (lowercase letters) or heat (uppercase letters) treatments are not significantly different at the 5% level by Tukey's HSD

Fig. 5 Expression levels of two *S. exigua* heat shock protein genes at different developmental stages relative to expression levels in second instars. Means with the same lowercase (white bars) or uppercase (black bars) letter are not significantly different by Tukey's HSD ($P>0.05$)

out (Fig. 6). Expression of *Se-Hsp70* was hardly detected or very weak at normal growth temperature of 26°C (Control), whereas it was very tense at low or high temperatures. Densitometric analysis of immunoblots showed that there were significant difference in expression of *Se-Hsp70* among treatments ($F_{(4, 10)}=8.130$, $P<0.05$). Higher expression of *Se-Hsp70* occurred at heat shock (43 and 45°C) compared with cold shock (−10 and 0°C). The highest value was appeared in larvae which were shocked at 43°C, followed by 45°C, but there were no significant difference between them. At −10°C, larvae expressed significantly more *Se-Hsp70* than those kept at 26°C, but there were no significantly different from those shocked by 0°C.

Fig. 6 Western blot showing expression profiles of *Se-Hsp70* protein induced by cold (−10 and 0°C) and heat (43 and 45°C) in fourth instar larvae of *S. exigua*. Hsp70 expression values are expressed as percentages of the highest value, which was set as 100. Data are presented as mean $\pm$ SEM from three independent experiments. Means with the same letter are not significantly different at the 5% level by Tukey's HSD

Similar results were obtained from expression profiles of *Se-Hsp70* protein induced by cold (0°C) and heat (43°C) in pupae (Fig. 7), which showing increased inducible *Se-Hsp70* expression levels regardless of cold or heat shock ($F_{(2, 6)}=6.374$, $P<0.05$). Interestingly, obvious *Se-Hsp70* expression level was observed in pupae even at normal growth temperature of 26°C.

Levels of *Se-Hsp70* protein varied greatly during development without thermal stress (Fig. 8; $F_{(4, 10)}=548.192$, $P<0.05$). *Se-Hsp70* was hardly detectable in larval development including newly hatched, third and fifth instar larvae. However, abundant levels of *Se-Hsp70* protein appeared to be present in pupae and were maintained through to the adults, although it decreased significantly from pupae to adults.

Fig. 7 Western blot showing expression profiles of *Se-Hsp70* protein induced by cold (0°C) and heat (43°C) in pupae of *S. exigua*. Hsp70 expression values are expressed as percentages of the highest value, which was set as 100. Data are presented as mean $\pm$ SEM from three independent experiments. Means with the same letter are not significantly different at the 5% level by Tukey's HSD

Fig. 8 Western blot showing expression of *Se*-Hsp70 protein at different developmental stages of *S. exigua*. Hsp70 protein level was examined at newly hatched larva, the third instar larva, fifth instar larva, pupa, and adult. Hsp70 expression values are expressed as percentages of the highest value, which was set as 100. Data are presented as mean $\pm$ SEM from three independent experiments. Means with the same letter are not significantly different at the 5% level by Tukey's HSD

Discussion

The beet armyworm, *S. exigua* is well known as a cosmopolitan pest with high environmental tolerance, causing severe damage in a variety of crops. HSPs play important roles in the environmental adaptation of various organisms. To explore the response of HSPs to thermal stress and changes during development in *S. exigua*, two full-length cDNAs HSP genes were cloned by an RT-PCR and RACE strategy. Transcriptional expression in response to cold and heat shock during development was investigated by real time quantitative PCR. Although inducible *hsp70* and constitutive *hsc70* have a high degree of amino acid sequence similarity in insects, 15 characteristic residues can reliably distinguish them (Chen et al. 2006). Based on the deduced amino acid sequence we observed, the *S. exigua* gene is an inducible *hsp70*. Thus, we named it *Se-hsp70*. Like HSP genes cloned and characterized from other insects, the coding regions of *Se-hsp90* and *Se-hsp70* were highly conserved. Several special motifs were found, including the EEVD tetrapeptide located at the C termini of both *Se-hsp90* and *Se-hsp70*, which may form a structure mediating cofactor binding (Fuertesa et al. 2004).

Although heat shock was the first stress shown to elicit synthesis of HSPs, it is now evident that a number of environmental, physiological and genetic factors (Sørensen et al. 2003), even cold shock (Goto and Kimura 1998), can regulate expression of these same proteins. Therefore, regulation of inducible HSPs plays an important role in cellular response. HSP function in thermotolerance has been confirmed in various organisms (Sonna et al. 2002; Huang and Kang 2007). In *S. exigua*, both *Se-hsp90* and

Se-hsp70 were positively induced during development when the insect was stressed, regardless of heat or cold, suggesting that up-regulation of these genes is essential for *S. exigua* thermotolerance.

The onset temperature, whether low or high, at which HSP gene expression is up-regulated varies between organisms (Garbuz et al. 2003). Tomanek and Somero (1999) have suggested the threshold temperature may be a useful way to identify temperature tolerance limits, and that the higher the onset temperature, the more heat tolerant an organism is, and vice versa. Findings in *Drosophila* (Garbuz et al. 2003) and leafminer species (Huang and Kang 2007) support this idea. In *S. exigua*, the relatively high onset temperature of 41 and 43°C for *Se-hsp90* and *Se-hsp70* in response to heat shock, respectively, is perhaps an indicator of heat tolerance in this species. In contrast, the not very low values of -10°C for both *Se-hsp90* and *Se-hsp70* under cold shock, respectively, may suggest only weak cold-tolerance in this species. Furthermore, the difference in onset temperatures between the two genes under heat shock—41°C in most developmental stages for *Se-hsp90* and 43°C in all tested development for *Se-hsp70*—may reflect different functions. That is, *hsp70* inducible expression at “normal” temperatures in organisms may play a negative role in cell growth and division, but not the *hsp90* (Feder et al. 1992). Expression of *Se-hsp70*, which was not significantly different across a wide temperature range from 26 to 43°C, is consistent with the negative role of *hsp70* overexpression on growth, survival and fecundity observed in other insects (Krebs and Loeschcke 1994; Huang et al. 2007). The onset temperature needed for *hsp70* expression is usually higher than that needed for *hsp90* expression (Huang and Kang 2007). Therefore, the absence of *Se-hsp70* expression at temperatures less than 43°C may indicate that *S. exigua* can tolerate a wider than “normal” temperature range, consistent with its high heat tolerance in the field.

Intensities of temperature response also vary among HSP genes in insects. *Hsp70* responds dramatically to either heat or cold shock, which can induce a thousand-fold response (Velazquez et al. 1983; Huang and Kang 2007). The massive boost of synthesis by thermal stress suggests a critical role for this protein in preventing cell injury, yet it is also essential that this inducible protein be absent at normal temperatures (Feder et al. 1992). This implies that HSP70 is the most predominant contributor to thermotolerance in insects. In *S. exigua*, the temperature responses of *Se-hsp70* were more intense than those of *Se-hsp90*, regardless of inducing temperature or developmental stage, also indicating *Se-hsp70* plays a more important role in thermotolerance. However, the overall intensities of response in either *Se-hsp90* or *Se-hsp70* at high temperatures were higher than those at low temperatures in larvae. Increases

from 4.5- to 6.9-fold in *Se-hsp90* and 494.0- to 2,733.3-fold in *Se-hsp70* were observed at high temperatures, respectively, while low temperatures induced only 1.6- to 5.9-fold and 78.4- to 549.5-fold increases (Figs. 3 and 4). This may reflect the reason why *S. exigua* larvae are more resistant to heat than cold in the field (Jiang and Luo 1999). Similar observations have been observed for two leafminer species (Huang and Kang 2007).

Finally, the basal HSP gene expression profiles varied with developmental stage, suggesting these genes may be involved in development (Feder and Hofmann 1999; Sonoda et al. 2006; Huang et al. 2009). Interestingly, the relative accumulated amounts of mRNA from large HSP genes, including *hsp90* and *hsp70*, may increase or decrease as larval and pupal development progresses, depending on the species (Mahroof et al. 2005a; Sonoda et al. 2006; Huang et al. 2009). In *S. exigua*, the relative transcript levels of both *Se-hsp90* and *Se-hsp70* decreased as development progressed. These results are consistent with research conducted on the red flour beetle, *Tribolium castaneum* (Mahroof et al. 2005a), which indicated that the mRNA level of *hsp70*III decreased with development of young larvae, old larvae, and pupae. Furthermore, induced HSP gene expression profiles also varied after heat or cold shock during development. Mahroof et al. (2005ab) suggested that increased mRNA abundance induced under thermal stress during different developmental stages may be contributing to the increased thermotolerance at those stages. Increased thermotolerance in young larvae of *T. castaneum* could be due to increased expression of *hsp70* (Mahroof et al. 2005a). In *S. exigua*, higher *Se-hsp90* and *Se-hsp70* expression was observed in heat shocked young larvae and cold shocked second instars and pupae, consistent with high heat resistance in larvae and a stronger cold tolerance in young larvae and pupae. These deduced *S. exigua* heat or cold tolerances during development based on HSP gene expression levels are in good agreement with thermotolerances observed in field populations (Ma et al. 1999; Jiang et al. 2001).

To further investigate *Se-Hsp* expression profiles at protein level either after thermal stress or during different developmental stages, Western blots using antibody to *Se-Hsp70* were detected in the fourth instar larvae and pupae after heat or cold shock, as well as in the larvae, pupae and adults without thermal stress. Generally, there are obvious positive correlation between *Se-Hsp70* expression and *Se-hsp70* RNA. First, lower *Se-Hsp70* protein expression in larvae and pupae at normal growth temperature of 26°C is consistent with corresponding lower *Se-hsp70* RNA expression at the same conditions. Second, increased *Se-Hsp70* expression in larvae and pupae under heat or cold shock treatment is in good agreement with the corresponding increased *Se-hsp70* RNA expression at the

same conditions. Moreover, the intensities of the inducible *Se-Hsp70* expression after different temperatures stress are always consistent with those which induce *Se-hsp70* RNA. These results indicated that increased *Se-Hsp70* expression under thermal stress is due to an increase of the *Se-hsp70* RNA. Positive relationship between *Hsp70* protein expression and *hsp70* gene expression always occurs at other insect species (Kalosaka et al. 2009).

However, *Se-Hsp70* protein expression profile during development without thermal stress does not completely accord with that of *Se-hsp70* RNA expression. Although *Se-hsp70* RNA decreased as development progressed, showing the higher expression occurs at young larvae, *Se-Hsp70* proteins at this stage are hardly detectable. This conflict between heat shock RNAs and Hsps usually exist in other species (Garbuz et al. 2008). Interestingly, Western blot results showed that the inducible *Se-Hsp70* protein is stably present at higher level in pupae in the absence of thermal stress, also, adults of the species exhibits visible amounts of *Se-Hsp70* protein at normal temperature, thus eliciting a significant importance of the regulation mechanism underlying transcription and translation for these HSPs during development in insects.

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