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ORIGINAL ARTICLE

Functional analysis of two *SfHsp90* genes in response to high temperature and insecticide stress in *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract. *Spodoptera frugiperda*, a worldwide pest, can feed on 353 crops species, including corn, rice, and sorghum. It is highly adaptable to various environments. Heat shock protein 90 kDa (Hsp90) plays a crucial role in the environmental adaptation of insects. To explore the role of *SfHsp90* genes coding for Hsp90 proteins in the high temperature and insecticides stress resistance of *Spodoptera frugiperda*, we identified the complete complementary DNA sequences of two *SfHsp90*s. Both of them were expressed at different developmental stages and tissues in *S. frugiperda*. The expression levels of the *SfHsp90*s were significantly upregulated when exposed to durations of extreme temperature (45°C) and lethal concentrations of emamectin benzoate (LC_{10} and LC_{20}). The viability of *S. frugiperda* under 45°C and emamectin benzoate stresses was examined. The mortality rate of *S. frugiperda* was significantly increased when subjected to 45°C and emamectin benzoate after knockdown of *SfHsp90*s by RNAi. These results suggest that *SfHsp90*s are essential for the resistance of *S. frugiperda* to high temperature and emamectin benzoate stresses.

1. INTRODUCTION

Spodoptera frugiperda (Lepidoptera: Noctuidae) is an agricultural pest native to the tropical and subtropical regions of the Americas. It is a worldwide pest that can migrate over long distances (Todd & Poole, 1980; Zhang & Wu, 2019). The larvae can feed on 353 host plant species in 76 plant families including corn, rice, sorghum and cotton, and when damage is serious, the land experiences total crop failure (Montezano et al., 2018). The substantial damage caused by *S. frugiperda* is associated with the species' strong adaptability to various environmental factors and its resistance to pesticides. For instance, under low temperature stress, *S. frugiperda* will produce a large amount of glycerol in the body, thereby reducing the damage (Vatanparast & Park, 2022). Under both high and low temperatures stress, the larvae of *S. frugiperda* exhibit strong tolerance (Tao et al., 2023). Furthermore, *S. frugiperda* has grown resistant to the primary chemical groups, including benzoylurea, carbamates, pyrethroids, and organophosphates, as a result of an overreliance on chemical insecticides (Carvalho et al., 2013; Gutiérrez-Moreno et al., 2019; Boaventura et al., 2020; Lira et al., 2020).

Hsps are a class of highly conserved molecular chaperone proteins that are involved in maintaining and regulating the conformation and function of various cellular proteins (Feder & Hofmann, 1999; King & MacRae, 2015). One of the strategies used by insects to adapt to changing environments is the regulation of Hsp expression (Rinehart et al., 2007; Zhang & Denlinger, 2010). Hsps can be induced under various biological stresses, including bacterial, viral and fungal stresses, as well as non-biological hazards such as high/low temperature, drug exposure, ultraviolet (UV) radiation and moisture (Rutherford et al., 2007; Zhai et al., 2010; Tungjitwitayakul et al., 2016). Hsps can be classified according to their molecular weight, structural characteristics, and functions, namely Hsp100, Hsp90, Hsp70, Hsp60, Hsp40, and small Hsps (Kampinga et al., 2009; Mayer, 2010). Hsp90 is a pivotal member of the heat shock

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protein family and is evolutionarily conserved, exhibiting high abundance in eukaryotic cells (Ohhara et al., 2021). Hsp90 contributes to the folding, maintenance of structural integrity, and proper regulation of a subset of cytosolic proteins (Gupta, 1995; Chen & Wagner, 2012; King & MacRae, 2015). Previous studies have demonstrated that Hsp90 is generally up-regulated by stresses, such as heat or cold shock, food deprivation, heavy metals and hyperosmotic stress (Rutherford & Lindquist, 1998; Chen et al., 2005; Sonoda et al., 2006; Chen & Wagner, 2012; Brom et al., 2015).

Insects are ectotherms with biological characteristics that are closely related to environmental factors such as high and low temperatures, and moisture, which can affect the growth, development, survival, and geographical distribution of insects (Bale et al., 2002; Chown & Terblanche, 2006; Wang et al., 2014), prompting them to respond to environmental changes (Deutsch et al., 2008; Bauerfeind & Fischer, 2014). Several studies have shown that the expression of *Hsp90* genes in insects has a regulatory effect under temperature stress. For instance, both high and low temperatures can induce the expression of *LsHsp90* genes in *Ladelphax striatellus* (Zhang et al., 2014). In addition, high temperature stress and insecticides can induce the expression of *Hsp90* genes in *Frankliniella occidentalis* (Wang et al., 2014), *Oligonychus coffeae* (Roy et al., 2016), *Panonychus citri* (Tian et al., 2015), and *Leptinotarsa decemlineata* (Dumas et al., 2019). Similarly, chemical pesticides are also an important factor that affects the lives of insects. The treatment of *Ostrinia furnacalis* larvae with deltamethrin, malathion, and carbaryl reduced the positioning abilities of males and behavioral responses in a wind tunnel, and the mating success rate of adults was also significantly reduced (Wei & Du, 2004). Duncan (2005) found that benzoquinone antibiotics could induce the expression of *Hsp90* in *Drosophila*. The expression of *BcHsp90* in *Bactrocera cucurbitae* was significantly higher in specimens exposed to long-term abamectin stress than those in the control group (Jiang et al., 2019). The *Alhsp90* genes of *Apolygus lucorum* were significantly upregulated under the treatment of cyhalothrin, imidacloprid, chlorpyrifos, and emamectin benzoate (Sun et al., 2014).

On the other hand, the molecular mechanism by which *S. frugiperda*'s *Hsp90* genes respond to high temperatures and insecticide stress remains unclear. In the present study, we cloned two *SfHsp90* genes from *S. frugiperda* and characterized their nucleotide sequences. The expression of *SfHsp90*s levels were examined across time and space, and in response to high temperature and insecticide stress. In addition, the role of Hsp90 in the response of *S. frugiperda* to high temperature and insecticide stress was studied using RNA interference (RNAi) technology. The expression of those two *SfHsp90*s is discussed in the context of high temperature and insecticide resistance, which aid in understanding the mechanisms of environmental tolerance in *S. frugiperda*.

2. MATERIALS AND METHODS

2.1 Insects

S. frugiperda specimens were originally collected from Guizhou, China (106°38′44″E, 26°26′13″N). The larvae were fed with corn leaves and artificial feed (Pinto et al., 2019), and the adults were fed with 10% honey water. Insects were reared in an artificial climate box, at a temperature of 25 ± 1°C, relative humidity 75% ± 5%, and photoperiod 16L : 8D.

2.2 Transcript expression of *SfHsp90* genes under different developmental stages and different tissues

Samples were collected at different developmental stages: eggs (n = 200), 1st-6th instar larvae (comprising first instar larvae; n = 100), second instar larvae (n = 60), third instar larvae (n = 30), fourth instar larvae (n = 10), fifth instar larvae (n = 5), sixth instar larvae (n = 3), 3-day-old pupae (n = 3), and adults (female and male; n = 3). In the tissue specificity experiment, 2-day-old third instar larvae (n = 100) of *S. frugiperda* were used to dissect tissues on ice, including the head, epidermis, midgut, hindgut, malpighian tubule and fat body. Each treatment included three biological replicates (N = 3). All samples were immediately frozen in liquid nitrogen and stored at −80°C.

2.3 High temperature and emamectin benzoate treatments

During temperature treatments, 2-day-old third instar larvae *S. frugiperda* were exposed to 45°C temperatures for 0 (control), 30, 60, 90, 120, and 150 min in a temperature-controlled climate chamber, after which they were immediately frozen in liquid nitrogen and stored at −80°C. Three biological replicates (N = 3) were used for all treatments and twenty samples (n = 20) were taken from each replicate.

Emamectin benzoate treatment involved soaking maize leaves for 20 s in 0 (CK), LC_{10} (0.005 mg/L) and LC_{20} (0.007 mg/L) treatment solutions, then drying, and feeding to 2-day-old third-instar larvae of *S. frugiperda* for 24 h, after which they were immediately frozen in liquid nitrogen and kept at −80°C. Three biological replicates (N = 3) were used per treatment and twenty samples (n = 20) were taken from each replicate. Sub-lethal concentration screening: Emamectin benzoate (Purity 95.6%) was diluted to 0.05, 0.025, 0.0125, 0.00625 and 0.003125 mg/L with acetone, respectively. The corn leaves were soaked in each dilution for 20 s, dried, and then arranged in plastic petri dishes. A third instar larva was starved for two hours in each petri dish. Three biological replicates (N = 3) were used per concentration and thirty samples (n = 30) were taken from each replicate, and the leaves of the control group were treated with acetone. The concentrations causing 10% and 20% mortality (LC_{10} and LC_{20}) values were estimated by Probit analysis (Polo-PC LeOra Software).

2.4. RNA interference

SfHsp90-1 and *SfHsp90-2* fragments were cloned into the pMD19-T Vector (Takara) and then transformed into *E. coli* DH5α Competent Cells (Takara). The plasmid was extracted, verified using Sanger sequencing, and subsequently used for Double-stranded RNAs (dsRNAs) synthesis using the 5×MEGA script T7 Kit (Invitrogen, USA). A green fluorescent protein (GFP) dsRNA (dsRNA-GFP) was used as an exogenous control (negative control).

The formulations ds*SfHsp90-1*, ds*SfHsp90-2* and ds*GFP* were prepared separately. Next, dsRNA or ds*GFP* with 1 μL concentration of 3000 ng was injected into the back intersegment of 2-day-old third instar larvae using a microsyringe (Shengong Biotechnology, China). After silencing the *SfHsp90-1* and *SfHsp90-2*

genes for 24 h, the mortality of *S. frugiperda* was assessed following exposure to emamectin benzoate for 24 h and 45°C for 2 h, respectively. Three biological replicates (N = 3) were used per treatment and forty samples (n = 40) were taken from each replicate.

2.5. Identification and analysis of *S. frugiperda Hsp90* genes sequences

Total RNA was extracted from *S. frugiperda*, cDNA was generated and amplified using PCR, and the product was purified, cloned, and sequenced using established protocols (Ruan et al., 2022). *Hsp90-1* and *Hsp90-2* gene sequences were identified based on the transcriptome database of *S. frugiperda* obtained using sequencing. Specific primers were designed using Primer 5.0 (Table 1). The full-length open reading frames (ORFs) of the two genes were confirmed using polymerase chain reaction (PCR).

ORFs were identified using the ORF finder graphical analysis tool (https://www.ncbi.nlm.nih.gov/orffinder/). Multiple sequence alignments were generated using the DNAMAN 8.0 sequence analysis software. Molecular weights, isoelectric points, instability indices, and domains were predicted using the ExPASy Molecular Biology Server (https://www.expasy.org/). Phylogenetic analysis was performed using MEGA 7.0 software with the neighbor-joining algorithm (1000 replications; Kumar et al., 2016).

2.6 Real-time quantitative PCR

Total RNA was extracted from each sample using the Eastep® Super Total RNA Extraction Kit (Shanghai Promega Biological Products, Ltd., Shanghai, China). cDNA was generated with 1 µg of total RNA using a HiFiScript cDNA Synthesis Kit (CoWin Biosciences). Real-time quantitative PCR (RT-qPCR) was performed using an established protocol (Ruan et al., 2022). Gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2011), and the ribosomal protein L27 (*RPL27*) and ribosomal protein L7 (*RPL7*) genes were selected as internal reference genes. Primers for the two *SfHsp90*s used for qRT-PCR were designed online using Primer-BLAST on the NCBI website (https://www.ncbi.nlm.nih.gov/tools/primer-blast/; Table 1).

Table 1. Primers for the *SfHsp90* genes of *S. frugiperda*.

Primer name	Primer sequence	Primer usage
Hsp90-1-F1	TAGCCTGCGGGTACTGGATT	Sequence amplification
Hsp90-1-R1	TCACGACTCACGTTTAGCGG	
Hsp90-1-F2	GACCGACCACATCAAGCTCT	
Hsp90-1-R2	ACGGAACGTTCACTCAGTCG	
Hsp90-2-F1	GAAATGCAGACCGATGTCGC	
Hsp90-2-R1	AGGGATAAGGTCCTCGCAGT	
Hsp90-2-F2	TGCGATTCCACGCGATCTTA	
Hsp90-2-R2	GCTCGTCGTCATTGTGCTTC	
Hsp90-2-F3	TTGAGAAGGAACGCGAGAAGG	
Hsp90-2-R3	CCAGTGCACAAAATAACTTCGC	
q*Hsp90-1*-F	CGTCTTCATCACCGACGAGT	Real-time PCR quantification
q*Hsp90-1*-R	TCACGACTCTCGTTTAGCGG	
q*Hsp90-2*-F	ACCTTGGTACCATCGCCAAG	
q*Hsp90-2*-R	CCACATGTACTGCTCGTCGT	
RPL27-F	GTGTCCGTAGGGCTTGTCTG	Reference gene
RPL27-R	GAAGCCAGGTAAAGTGGTGCT	
RPL7-F	TTCGTCATCCGTATCCGTGG	
RPL7-R	ATGTAGGGCTCGGCAATACG	
ds*SfHsp90-1*-F	CGCGCCCAGAACTATACATT	dsRNA synthesis
ds*SfHsp90-1*-R	CATGTCGTTCATCTCTTGCG	
ds*SfHsp90-2*-F	AAGTTCGAGGGTCTCTGCAA	
ds*SfHsp90-2*-R	TCGTACAGCAGGATGACGAG	
ds*GFP*-F	GCCAACACTTGTCACTACTT	
ds*GFP*-R	GGAGTATTTTGTTGATAATGGTCTG	

2.7 Data analysis

All data were tested for variance homogeneity using SPSS 20.0 (IBM, Corporation, Armonk, NY, USA). Values from qRT-PCR data (*SfHsp90-1* of developmental stage, *SfHsp90-2* of high temperature, and *SfHsp90-2* of emamectin benzoate treatments) were natural-log-transformed to increase homogeneity of variance. The data were analyzed using one-way analysis of variance (ANOVA) in SPSS 20.0. Multiple comparisons and analyses were performed using Tukey's method. A P value of < 0.05 was considered significant.

3. RESULTS

3.1 Cloning and sequence analysis of the *SfHsp90* genes

Two *SfHsp90* genes (*Hsp90-1*, *Hsp90-2*) from *S. frugiperda* were cloned, with GenBank accession numbers MW990251 and MW990252. Sequence analysis revealed that the two genes included ORFs of 2364 and 2154, encoding 787 and 717 amino acids, respectively. The predicted molecular weights of SfHsp90s were 89.34 and 82.52 kDa, theoretical isoelectric points were 4.92 and 5.01, and instability indices were 40.94 and 39.23.

The results of the ExPASy PROSITE analysis indicated that two SfHsp90s contain an Hsp90 family signature sequence, YRNKEIFLRE/YSNKEIFLRE, and the amino acid terminal sequence contained the HDEL and EEVD motifs (Fig. 1), indicating that they were endoplasmic reticulum-type and cytoplasmic-type heat shock proteins. The phylogenetic tree showed that the two SfHsp90s of *S. frugiperda* were clustered with Hsp90 of Lepidoptera, whereas Hsp90 from Coleoptera, Hymenoptera, and Hemiptera fell into separate clades (Fig. 2).

3.2. Expression levels of the two *SfHsp90* genes at different developmental stages in *S. frugiperda*

The mRNA expression levels of the two *SfHsp90* genes at different developmental stages were analyzed using qRT-PCR. The genes were expressed in all developmental stages, but the expression pattern varied based on the stage. *SfHsp90-1* expression was the highest in 6th and 3rd instar larvae, and the lowest in pupae ($F_{9, 20} = 1483.683$, $P < 0.001$). The expression level of *SfHsp90-2* was the highest in the 3rd instar larvae and the lowest in the 5th instar larvae ($F_{9, 20} = 1753.690$, $P < 0.001$; Fig. 3).

3.3. Expression levels of the two S*fHsp90* genes in different *S. frugiperda* tissues

The expression levels of the two *SfHsp90* genes were significantly different in 6 tissues of the 3rd instar larvae of *S. frugiperda*. The expression levels of *SfHsp90-1* were highest in the epidermis ($F_{5, 12} = 428.947$, $P < 0.001$), whereas *SfHsp90-2* expression levels were highest in the fat body ($F_{5, 12} = 508.319$, $P < 0.001$). In contrast, the expression levels of *SfHsp90-1* and *SfHsp90-2* were lowest in the hindgut (Fig. 4).

3.4. Expression levels of the two *SfHsp90* genes under high temperature stress at 45°C

Under high temperature stress at 45°C, the expression levels of the two S*fHsp90* genes varied significantly. The

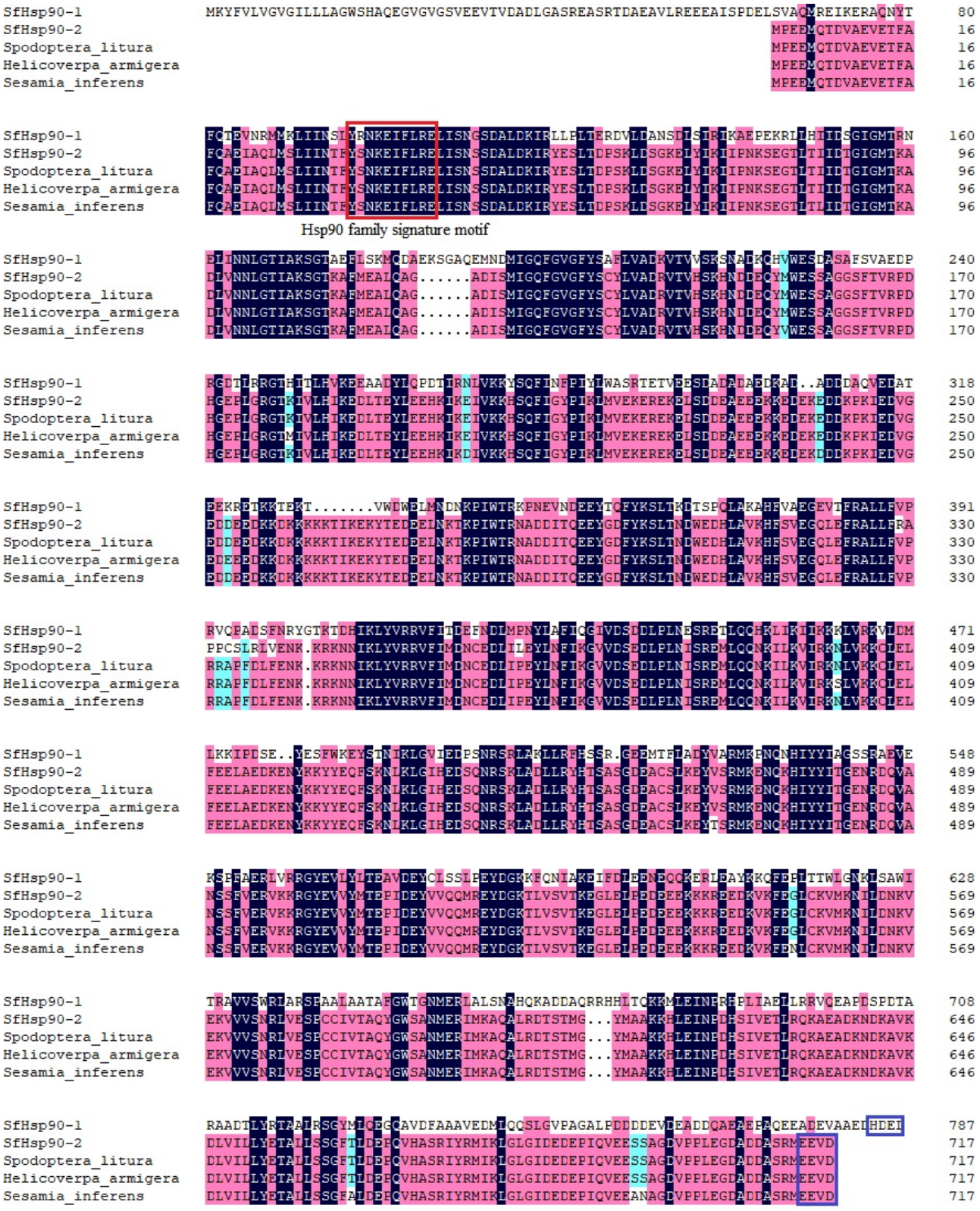

Fig. 1. Multiple sequence alignment of SfHsp90s from *S. frugiperda*. The red box indicates the Hsp90 family signature motif. The blue boxes indicate that they are endoplasmic reticulum-type and cytoplasmic-type heat shock proteins.

expressions of *SfHsp90-1* and *SfHsp90-2* increased initially and then decreased over time, and reached the peak value at 60 min, which was significantly higher than that of the control group (0 min; $F_{5, 12} = 188.719$, $P < 0.001$; $F_{5, 12} = 1303.772$, $P < 0.001$; Fig. 5).

3.5. Expression levels of the two *SfHsp90* genes during emamectin benzoate treatment

Under 24 h of emamectin benzoate treatment (LC_{10}; LC_{20}), the expression levels of both *SfHsp90* genes were significantly increased. The peak values reached at LC_{20}, at

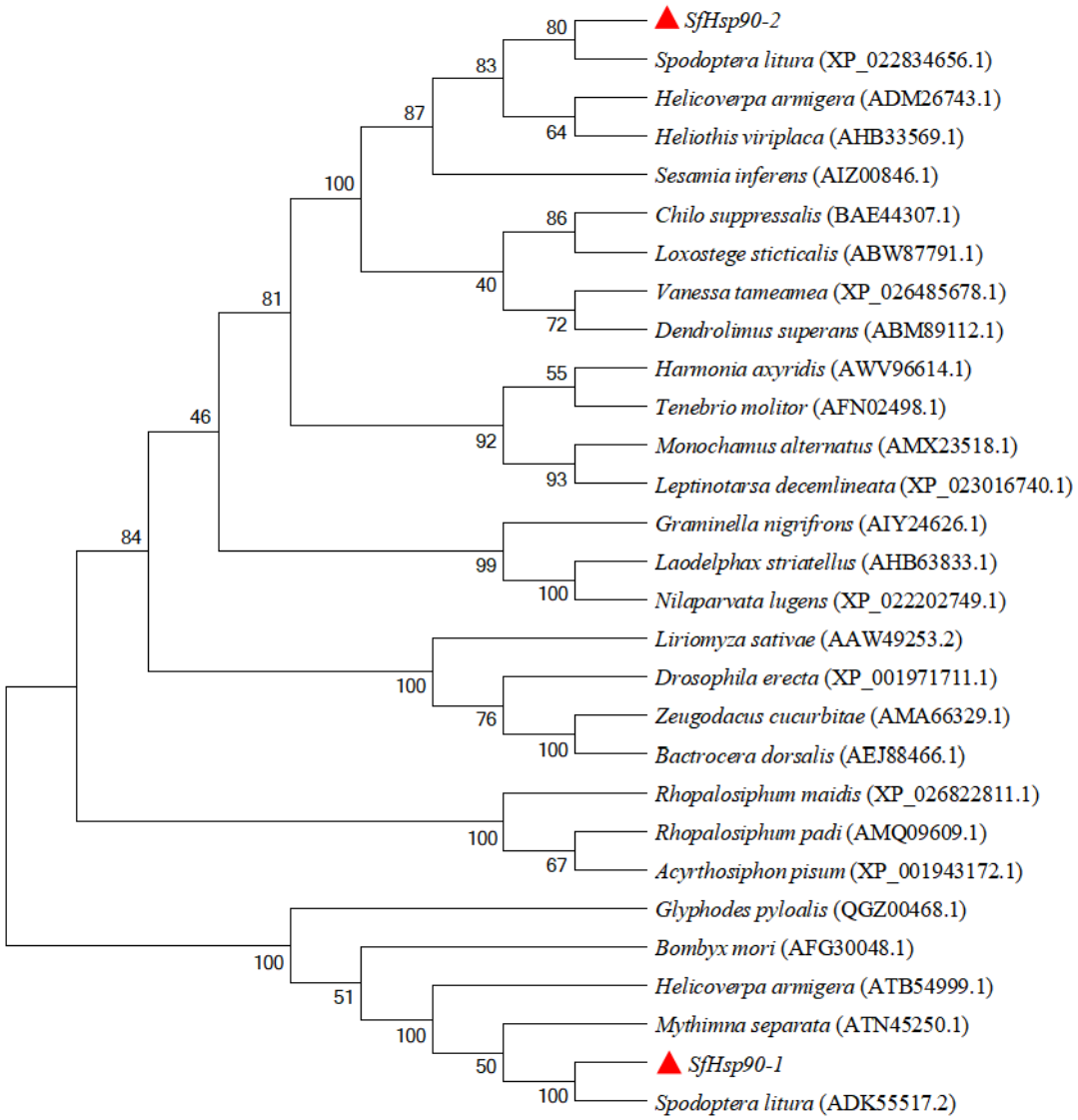

Fig. 2. Neighbor-joining phylogenetic tree of Hsp90s of selected *S. frugiperda*. Numbers on the branches are bootstrap values obtained from 1000 replicates. Sequence labels are indicated by the species names and GenBank accession numbers. Red triangles represent the sequences of *S. frugiperda* Hsp90s.

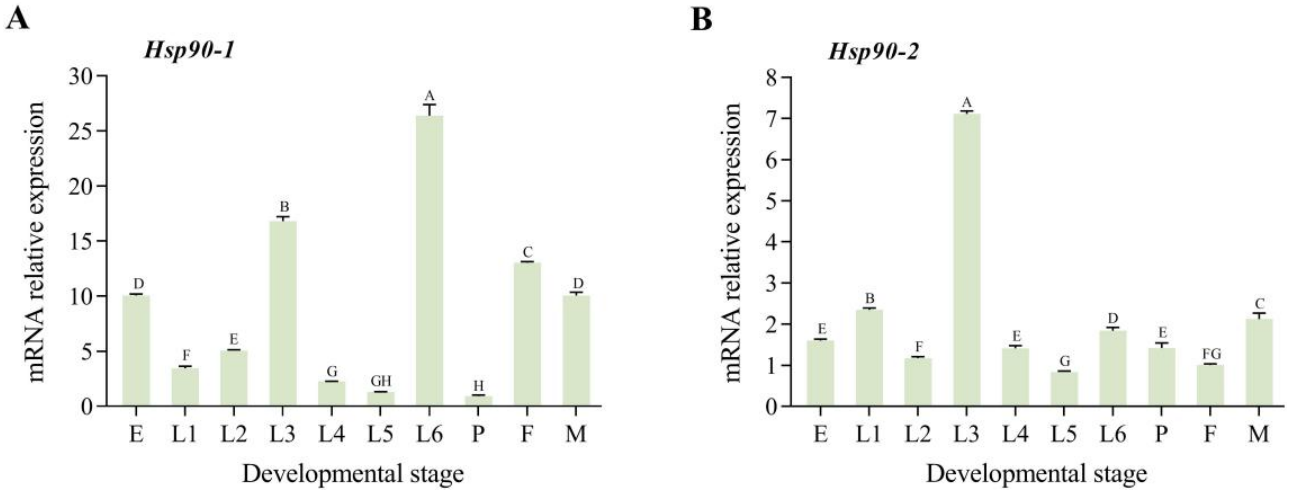

Fig. 3. Relative expression levels of two *SfHsp90* genes at different developmental stages in *S. frugiperda*. E – egg; L1–L6 – first to sixth instar larvae; P – pupa; FA – female adult; MA – male adult. Error bars showed the standard errors of the means of three biological replicates. Data are presented as mean ± standard error (N = 3). Signifcant differences are marked with letters ($P < 0.05$, one-way ANOVA).

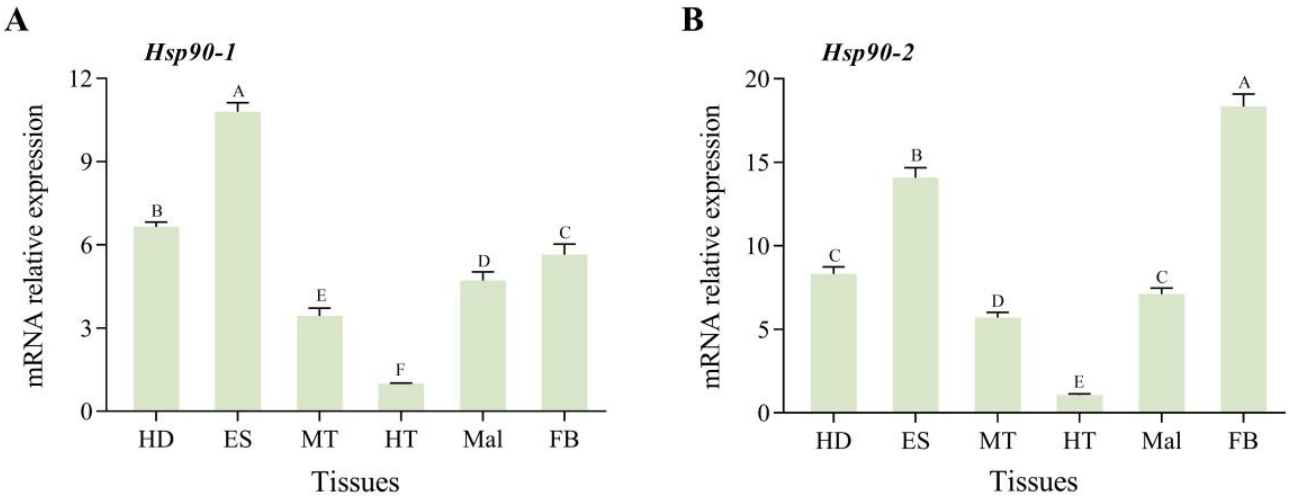

Fig. 4. Relative expression levels of the two *SfHsp90* genes in different tissues of the third instar larvae of *S. frugiperda*. HD – head; ES – epidermis; MT – midgut; HT – hindgut; Mal – malpighian tubule; FB – fat body. Error bars showed the standard errors of the means of three biological replicates. Data are presented as mean ± standard error (N = 3). Signifcant differences are marked with letters ($P < 0.05$, one-way ANOVA).

which points, expression levels were significantly higher than those in the control (0 min; $F_{2,6} = 3978.473$, $P < 0.001$; $F_{2,6} = 1357.727$, $P < 0.001$; Fig. 6).

3.6 Determination of RNAi silencing efficiency

The third instar larvae of *S. frugiperda* were injected with ds*Hsp90-1* and ds*Hsp90-2*, respectively, and the control group was injected with ds*GFP*. Samples were collected at 24 h for detection. The results indicated that the expression of *SfHsp90-1* in insects injected with ds*Hsp90-1* decreased by 51.67% and the expression of *SfHsp90-2* in insects injected with ds*Hsp90-2* decreased by 67.47% as compared to insects injected with ds*GFP* (Fig. 7).

3.7 Effects of *SfHsp90* silencing on *S. frugiperda* stress tolerance

After treatment at 45°C and with emamectin benzoate (LC_{20}), the mortality of the experimental group injected with ds*Hsp90s* was significantly higher compared with that of the control group injected with ds*GFP*. After exposure to 45°C stress, the mortality of *S. frugiperda* injected with ds*Hsp90-1* was 66.66%, and the the mortality of *S. frugiperda* injected with ds*Hsp90-2* was 64.17%, which was significantly higher compared with that injected with ds*GFP* (22.50%, Fig. 8A). After feeding with maize leaves (soaked with emamectin benzoate at an LC_{20}) for 24 h, the mortality of *S. frugiperda* following ds*Hsp90-1* injection was 78.33%, and the the mortality of *S. frugiperda* injected with ds*Hsp90-2* was 60.83%, which was significantly higher compared with those injected with ds*GFP* (20.83%, Fig. 8B).

4. DISCUSSION

In the present study, we successfully cloned two *SfHsp90* genes. Analysis using ExPASy PROSITE revealed that two *SfHsp90*s contained the Hsp90 family signature motif YRNKEIFLRE/YSNKEIFLRE, indicating that belong to the Hsp90 family. Similar results were obtained in studies of *Myzus persicae*, *B. cucurbitae*, and *Carposina sasakii*

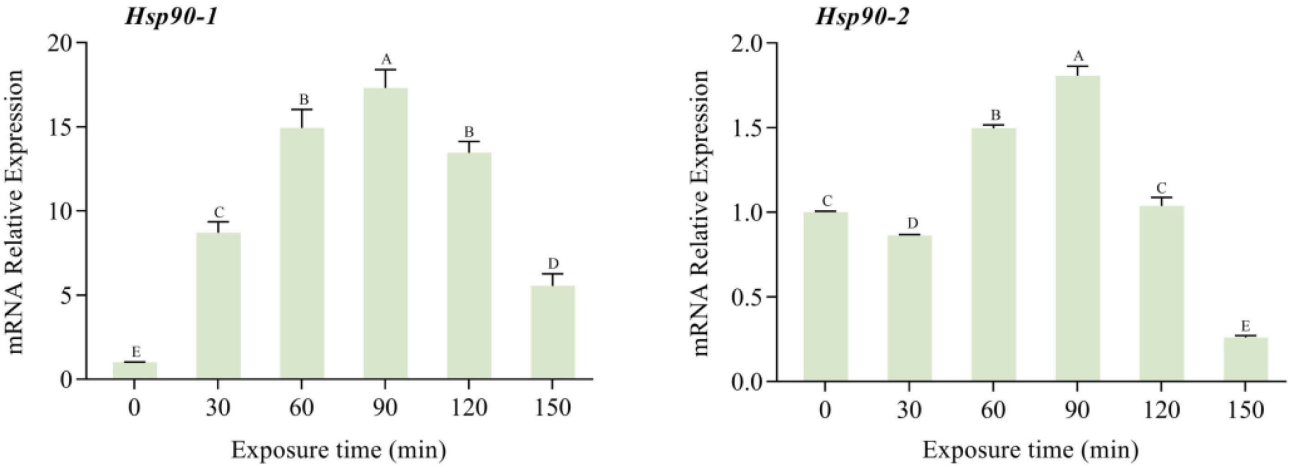

Fig. 5. Effect of high temperature (45°C) stress on the expression of two *SfHsp90* genes in *S. frugiperda*. Error bars showed the standard errors of the means of three biological replicates. Data are presented as mean ± standard error (N = 3). Signifcant differences are marked with different letters (P < 0.05, one-way ANOVA).

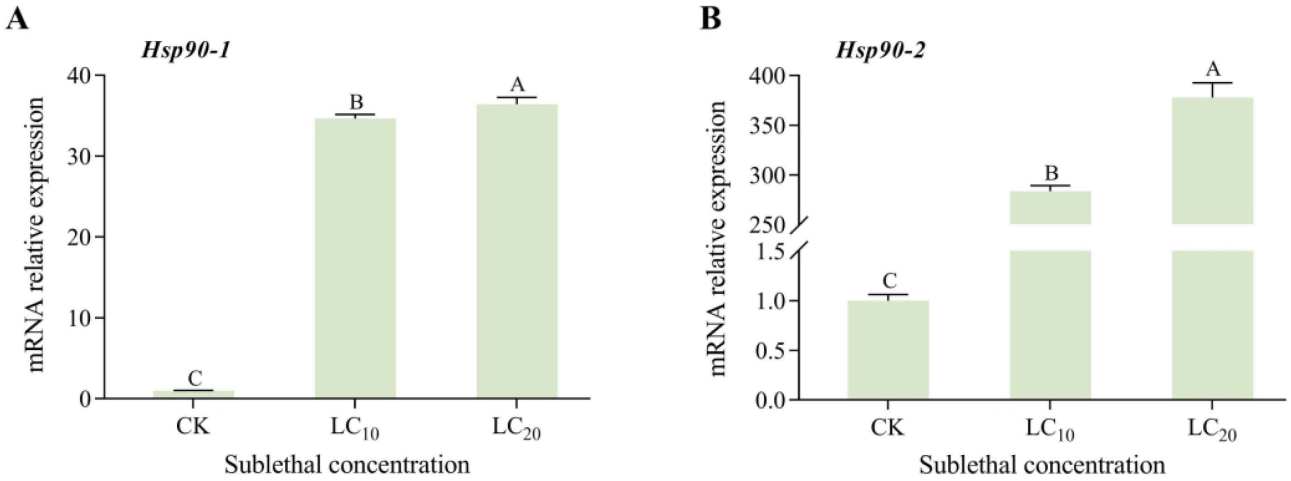

Fig. 6. Effect of emamectin benzoate treatment on the expression of two *SfHsp90* genes in *S. frugiperda*. Error bars showed the standard errors of the means of three biological replicates. Data are presented as mean ± standard error (N = 3). Signifcant differences are marked with different letters ($P < 0.05$, one-way ANOVA).

(Gao et al., 2016; Su et al., 2018; Jiang et al., 2019). The terminal amino acid sequence of SfHsp90s were HDEL and EEVD, which indicated that SfHsp90s exists in the endoplasmic reticulum and cytoplasm, respectively, and that they were endoplasmic reticulum-type and cytoplasmic-type heat shock proteins (Gupta, 1995). The phylogenetic tree showed that two SfHsp90s was clustered with Hsp90 of Lepidoptera, whereas Hsp90 from Coleoptera, Hymenoptera, and Hemiptera fell into a separate clade, indicating that Hsp90 in *S. frugiperda* is highly conserved during evolution. This result is similar to the clustering results of Hsp90 from *Cydia pomonella* and the Brown Planthopper (Shen et al., 2011; Chen, et al., 2020).

Various insect *Hsp90* genes have specific expression patterns at different developmental stages. For instance, the expression level of *PxHsp90* in *Plutella xylostella* was very low in fourth instar larvae, but it increases in pupae and peaks in adults. At the adult stage, females showed higher levels of mRNA expression than males (Sonoda et al., 2006). The expression of *Slhsp90* in *Spodoptera litura* ranged from the fifth instar larvae to adults. The highest level of expression was detected in adults, while the lowest level was detected in fifth instar larvae (Shu et al., 2011). The expression level of *MsHsp90* in *Mythimna separata* was higher in 2nd instar larvae in comparison with other developmental stages (Yang et al., 2017). In the present study, two *SfHsp90* genes were expressed at different developmental stages in *S. frugiperda*, but there were significant differences. The expression level of *SfHsp90-1* was highest in 6th and 3rd instar larvae and the expression level of *SfHsp90-2* was highest in 3rd instar larvae. These results suggest that *Hsp90* genes are related to the growth and development of insects at different stages, and play an important role in the regulation of different developmental stages.

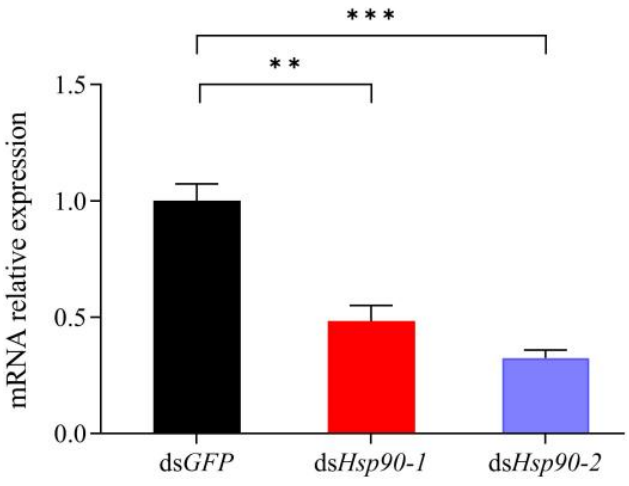

Fig. 7. The relative expression of two *SfHsp90* genes after RNA interference. Data are presented as mean ± standard error (N = 3). Asterisks indicate significant differences (Student's t-test, **P < 0.01, ***P < 0.001).

The expression patterns of *Hsp90* genes in insects suggest a level of tissue-specificity. For example, *Hsp90* genes were expressed significantly differently dependant on tissue in *Rhopalosiphum padi* (Li et al., 2017), *Anaphothrips obscurus* (Guo & Feng, 2018), brown planthopper (Chen et al., 2020), and *Sitophilus zeamais* (Tungjitwitayakul et al., 2015). In the present study, the two *SfHsp90* genes of *S. frugiperda* were expressed in six tissue types in 3rd instar larvae, in which their expression patterns also varied. Notably, the expression of *Hsp90-1* was highest in the epidermis and head, which may be because the head is the center of insect perception of external environmental changes, and the epidermis is the first barrier to cope with environmental stress. The expression of *Hsp90-2* was highest in the fat body and epidermis, which may be attributed to the epidermis serving as the primary defense against environmental stress, while the fat body plays an important role in regulating metabolism and enhancing cold tolerance in insects (Popović et al., 2015). Thus, *Hsp90* genes expressed in specific tissues may play important and unique roles in maintaining organismal functions.

The heat shock protein Hsp90 is closely associated with the environmental tolerance of insects, and a large number of studies have confirmed that environmental stress induc-

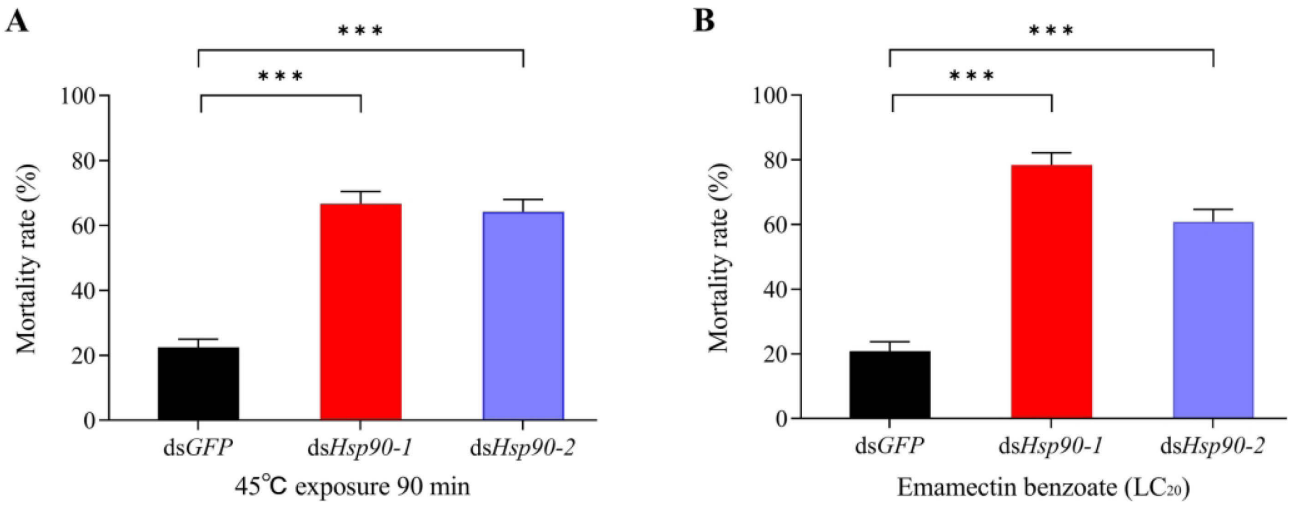

Fig. 8. RNAi in third instar larvae of *S. frugiperda*. (A) Mortality rate in third instar larvae of expose to 45°C on 90 min after inject ds*SfHsp90*s or ds*GFP* for 24 h. (B) Mortality rate in third instar larvae of expose to emamectin benzoate on 24 h after inject ds*SfHsp90*s or ds*GFP* for 24 h. Data are presented as mean ± standard error (N = 3). Asterisks indicate significant differences (Student's t-test, ***P < 0.001).

es *Hsp90* expression. For example, the expression level of *PmHsp90* in *Pieris melete* was significantly up-regulated at high and low temperatures of 39°C and 4°C, respectively (Wu et al., 2018). The expressed pattern observed in *Helicoverpa zea* indicates that *HzHsp90* is thermally induced, and that low temperatures can induce *HzHsp90* response (Zhang & Denlinger, 2010). After a 24-h exposure to dimethoate treatment, the expression level of *LdHsp90* in *L. decemlineata* significantly increased (Brom et al., 2015). In our research, the expression level of *SfHsp90* genes was significantly increased in *S. frugiperda* under high temperature stress, indicating that high temperatures can induce *SfHsp90* genes expression. This result is consistent with the findings obtained by Yang et al. (2017), who applied different temperatures in treatments on *M. separata*. The expression level of *SfHsp90* genes increased and then decreased with increasing treatment time. Similar results were found in *Sitodiplosis mosellana* and *M. separata*, suggesting a threshold for the protective effect of *Hsp90* on organisms (Cheng et al., 2016; Yang et al., 2017). In the present study, the expression level of *SfHsp90* genes was up-regulated in *S. frugiperda* after feeding on emamectin benzoate. Similar results were found in the expression of *BcHsp90*s on *B. cucurbitae* under abamectin stress (Jiang et al., 2019). Increased *AlHsp90*s levels were observed in *A. lucorum* exposed to cyhalothrin and imidacloprid (Sun et al., 2014). The results show that the *Hsp90* genes play an important role in tolerance to insecticide stress in *S. frugiperda*.

The heat stress protein Hsp90 can stabilize the cytoskeleton, affect the process of cell secretion and regulation, and enhance the tolerance of insects to environmental stress (Krishnamoorthy et al., 2007; Chen et al., 2010). To explore the function of SfHsp90 in more detail, we used RNAi technology to knock down the expression of the *SfHsp90* genes. The results indicated that after successful knockdown of *SfHsp90-1* and *SfHsp90-2*, the mortality of *S. frugiperda* significantly increased when exposed to emamectin benzoate and under temperature 45°C. Similar results were observed when *Glyphodes pyloalis* larvae were subjected to RNA interference to silence *GpHsp82.4*, which led to a significant increase in mortality under heat stress (Ding et al., 2021). RNAi-mediated knockdown of *CmHsp90* in carob moth larvae greatly reduced the ability of the insects to withstand extreme temperature treatments (Farahani et al., 2020). Thus, RNAi knockdown significantly reduced the tolerance to high temperature and emamectin benzoate in *S. frugiperda*, further suggesting that *SfHsp90*s play an important role in the adaptation of *S. frugiperda* to environmental stress. This study elucidated a role for *SfHsp90*s in response to environmental stress and provides a foundation for further exploring the molecular mechanism of *S. frugiperda* and it's adaptability to environmental stress.

5. CONCLUSION

In summary, *SfHsp90-1* and *SfHsp90-2* were identified and their expression patterns were determined at different developmental stages and in various tissues. They were significantly up-regulated both in response to high temperature and exposure to emamectin benzoate. The tolerance function of *SfHsp90*s in *S. frugiperda*'s response to high temperature and emamectin benzoate stress was examined by RNAi. Our findings highlight the significance of *SfHsp90*s of *S. frugiperda* in it's response to high temperature and insecticide stress, which is important for deciphering the molecular mechanism of its wide thermal tolerance range and high insecticide resistance.

AUTHOR CONTRIBUTIONS. All authors developed the project. Hong-Yun Ruan: Conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing-original draft preparation. Lv Zhou: methodology, validation, formal analysis, investigation, writing-review. Lei Yang and Jian-Yu Meng: supervision, writing-review. Chang-Yu Zhang: methodology, resources, writing-review and editing, project administra-

tion, funding acquisition. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT. The sequences derived from this study are deposit in Genbank accession numbers MW990251 and MW9902DATA.

CONFLICT OF INTERESTS. The authors declare that there are no confl ict of interests.

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