Molecular cloning of heat shock protein gene HSP90 and effects of abamectin and double-stranded RNA on its expression in *Panonychus citri* (Trombidiformes: Tetranychidae)

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Abstract

Panonychus citri McGregor (Trombidiformes: Tetranychidae), the widely distributed citrus red mite, has developed resistance to most registered acaricides. Adaptation of arthropods to extreme environmental conditions has been related to increased expression of their heat shock proteins (HSPs). The objectives of this study were to explore the relationship between HSPs and resistance of *Panonychus citri* to the acaricide abamectin and the adaptation of *Panonychus citri* to high temperatures. The full-length cDNA of the HSP90 gene was cloned from an abamectin-sensitive strain of *Panonychus citri*. This gene consisted of 2,495 nucleotides with a complete open reading frame (ORF) of 2,169 nucleotides. This gene encoded a polypeptide of 721 amino acids with a predicted molecular weight of 83.44 kDa, a theoretical isoelectric point of 5.06, a 3' untranslated region (UTR) of 228 bp, and a 5' UTR of 98 bp. The results of real-time PCR analyses indicated that the expression of the HSP90 gene in *P. citri* was markedly affected by the concentration of abamectin, the duration of exposure to it and the temperature, suggesting that the up-regulation of the HSP90 gene may play an important role in abamectin resistance and adaptation to high temperatures in *Panonychus citri*. The results of RNA interference experiments indicated that the HSP90 gene from adult female *Panonychus citri* was sensitive to down-regulation by double-stranded RNA (0.1–0.2 μg/**μL**). This study provides a molecular basis for further analysis of the relationships between the HSP90 gene and the resistance of *Panonychus citri* to abamectin and to high temperatures.

Key Words: citrus red mite; abamectin; HSP90; double-stranded RNA; resistance mechanism

Resumen

Panonychus citri McGregor (Trombidiformes: Tetranychidae), el ácaro rojo de los cítricos ampliamente distribuido, ha desarrollado resistencia a la mayoria de los acaricidas registrados. La adaptación de los artrópodos a condiciones ambientales extremas se ha relacionado con el aumento de expresión de sus proteínas de choque térmico (HSPs, en inglés). El objetivo de este estudio fue explorar la relación entre el HSP y la resistencia de *Panonychus citri* al acaricida, abamectina, y la adaptación de *Panonychus citri* a altas temperaturas. El ADNc de longitud completa del gen HSP90 fue clonado a partir de una cepa de *Panonychus citri* sensible de abamectina. Este gen consistió en 2,495 nucleótidos con un marco de lectura abierto completo (ORF) de 2169 nucleótidos. Este gen codifica un polipéptido de 721 aminoácidos con un peso molecular predicho de 83.44 kDa, un punto isoeléctrico teórico de 5.06, una región 3' no traducida (UTR) de 228 pb y una 5' UTR de 98 pb. Los resultados del análisis de PCR en tiempo real indicaron que la expresión del gen HSP90 en *P. citri* fue marcadamente afectada por la concentración de abamectina, la duración de la exposición a la misma y la temperatura, lo que sugiere que la sobre regulación del gen HSP90 puede jugar un papel importante en la resistencia a la abamectina y a la adaptación a las altas temperaturas en *P. citri*. Los resultados de los experimentos de interferencia de ARN indicaron que el gen HSP90 de la hembra adulta de *Panonychus citri* fue sensible a la baja regulación de ARN de doble cadena (0.1-0.2 g/l). Este estudio provee una base molecular para el análisis adicional de las relaciones entre el gen HSP90 y la resistencia de *Panonychus citri* a la abamectina y a altas temperaturas.

Palabras Clave: ácaro del rojo de los cítricos; abamectina; HSP90; ARN de doble cadena; mecanismo de resistencia

The citrus red mite, *Panonychus citri* McGregor (Trombidiformes: Tetranychidae), is a citrus pest widely distributed throughout the world, including in the citrus growing areas of China (Liu et al. 2011; Xia et al. 2014). *P. citri* punctures and feeds on the contents of cells,

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mainly of leaves, tender shoots, flower buds and fruit of citrus plants. The severe damage these mites cause induces loss of leaves, flowers and fruits and reduces citrus yield. Chemical control remains the most effective method for controlling *P. citri*. However, *P. citri* easily develops resistance to acaricides because of the mite's minuscule size, rapid production of generations and strong reproductive performance. *Panonychus citri* is resistant to most currently registered acaricides (Furuhashi 1994; Hu et al. 2010; Ouyang et al. 2012). The new antibiotic acaricide abamectin is effective against eggs, larvae and adult mites. Since abamectin was first introduced onto the Chinese market in 1991, it has played an important role in control of mite pests in China. Currently, more than 10 companies produce the active ingredient, abamectin, and approximately 300 organizations have registered abamectin formulations, of which approximately 140 enterprises have registered abamectin formulations to control *P. citri* (Chemical Book. 2010; China Pesticide Information Network). However, due to the long-term application of abamectin, many pest mite species have evolved resistance to it, and abamectin-resistant *P. citri* have appeared in some areas of citrus production (Zhang et al. 2013), hindering the sustainable control of this pest. Revealing the molecular mechanism of this resistance is crucial for coping with the resistance problem.

Heat shock proteins (HSPs) are rapidly generated in both prokaryotes and eukaryotes under environmental stress. The increase of HSPs in arthropods is related to their adaptation to extreme temperature, drought, radiation and pesticides (Feder & Hofmann 1999; Padmini 2010; Akerfelt et al. 2011; Zhu et al. 2013). A recent study indicated that the abamectin resistance in *Tetranychus cinnabarinus* (Boisduval) (Trombidiformes: Tetranychidae) may be associated with an increase in HSP90 gene expression (Feng et al. 2010). Some abamectin resistance has been generated in *P. citri*, but the molecular mechanism for this resistance remains unclear. Therefore, it is essential to elucidate the relationship between HSPs and abamectin resistance in *P. citri*.

RNA interference (RNAi) has been widely applied in the analysis of gene function (Fire et al. 1998; Campbell et al. 2010; Li et al. 2011). It is a post-transcriptional gene silencing process induced by double-stranded RNA (dsRNA), with gene expression inhibited by interference in the translation or transcription of specific genes. Due to the small size of individual mites, few studies have been conducted by employing RNAi technology in mites, and to date, no studies have applied RNAi technology to examine gene function in *P. citri*. Thus, we sought to establish an appropriate RNAi methodology to systematically analyze the function of HSPs in *P. citri*.

To determine the relationship between HSPs and the abamectin resistance of *P. citri*, as well as to examine the mechanism for the adaptation of *P. citri* to high temperatures, the complete HSP90 gene sequence from an abamectin-sensitive strain of *P. citri* was cloned. The influence of abamectin on the expression of the HSP90 gene in *P. citri* adult females by various abamectin concentrations and temperatures was analyzed. In addition, a methodology for silencing the HSP90 gene was developed using RNAi to systematically analyze the function of HSPs in *P. citri*.

Materials and Methods

EXPERIMENTAL MATERIALS

A relatively abamectin-sensitive *P. citri* mite strain, bred on a lemon tree in an abamectin-free field in the Citrus Research Institute of the Chinese Academy of Agricultural Sciences, was collected in 2013. The experimental population was established with lemon leaves as the host plant, and adult female mites (15 days old) were the experimental materials. *Panonychus citri* was reared indoors using the leaf disc method (Li et al. 2014). The culture dish was 9 cm in diam, and the dish was covered with a wet sponge of the same diam. An explanted fresh lemon leaf was placed over the sponge with the abaxial side facing upward, and the leaf margin was surrounded by a degreased cotton strand. This strain was maintained in an indoor laboratory at 25 ± 1 °C with 70–80% relative humidity (RH) and a 14:10 h light: dark cycle.

The 1.8% abamectin EC was purchased from Liuzhou Huinong Chemical Co., Ltd. (Liuzhou, Guangxi).

CLONING AND SEQUENCE ANALYSIS OF THE HSP90 GENE FROM *P. CITRI*

After the protein sequences of HSP90 from *Ixodes scapularis*, *Xenopus tropicalis*, *Chiromantes haematocheir*, *Tigriopus japonicus*, *Liriomyza sativae*, and *Equus caballus* were obtained from NCBI, 2 pairs of degenerate primers were designed with Primer Premier 5.0 to amplify the HSP90 fragment: HSP90-DF1 (5'-GGCACCAAGGCCTTCATGgargcnytnca-3'), HSP90-DR1 (5'-GCCCGGAACTCCAGCtgnccytcnac-3') and HSP90-DF2 (5'-GCCAAGTCCGGCACCAAGGCnttyatggarg-3'), HSP90-DR2 (5'-CATGATGAACACCCGCCGCacrtanarytt-3'). The same sequence was obtained while the middle fragment was compared with transcriptome database, and 2 specific primers were designed for each fragment: HSP90-5GSPR1 (5'-CCACCAGCCGAGGACTCCCACACAT-3'), HSP90-5GSPR2 (5'-TACCACGACCAAGGGGCTCACCAGT-3') and HSP90-3GSPF1 (5'-GAAGACAAGGGACAAGGAACTCAGCGA-3'), HSP90-3GSPF2 (5'-AAACCAAGCCTATCTGGACCCGCAAT-3').The sequences of the 5'-end and 3'-end of HSP90 gene from *P. citri* were amplified.

The total RNA of *P. citri* was extracted based on the instructions provided in the RNAprep Pure Micro kit (TIANGEN). The PrimeScriptRT reagent kit with gDNA Eraser (TaKaRa) was used to synthesize the cDNA. The PCR amplification was conducted according to the method of Staheli et al (2009). The amplification of the cDNA ends was conducted according to the instructions provided in the SMARTerRACE cDNA Amplification kit and the Advantage 2 PCR kit (Clontech).

DNA sequences were subjected to the BLAST tool on the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 5'- and 3'-end sequences were spliced with DNAMAN 5.0. The online software ORF Finder from NCBI (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to analyze the open reading frame of the complete HSP90 gene sequence in *P. citri*. Using Mega 4.0 and ClustalX, *P. citri* was compared with 4 insect/mite species showing high amino acid sequence homology to determine the degree of homology.

BIOASSAY METHOD

The slide impregnation method recommended by Food and Agriculture Organization of the United Nations was employed for treatment applications (Busvine 1980). Based on preliminary experiments, the abamectin solutions were prepared with 5–7 concentrations using a geometric series of mortalities (20%–90%) in *P. citri* adult females. Double-sided adhesive tape (3 cm) was adhered to one end of a clean slide. Healthy adult female mites were picked using a small brush and the dorsal side of the mite was affixed to the tape. Each slide held 30 individuals, and 4 replicates were used for each treatment. For the treatment group, the slides with the adhered mites were immersed in an abamectin solution, mildly agitated for 5 s and then removed. Mites in the control group were similarly treated but with clean water instead of abamectin. Excess solution on the surface of the *P. citri* females was removed with absorbent paper. The mites were then air-dried at room temperature for 15 min and placed into an illumination incubator (25 ± 1 °C, RH 70–80%). After 24 h, the mites were observed using a bin-

ocular lens, and the number of dead and living mites was recorded. Data were processed using SPSS 17.0 software. The toxicity regression equations were fitted, and the LC_{25}, LC_{50} and LC_{75} values of abamectin were calculated.

EFFECT OF ABAMECTIN CONCENTRATIONS AT VARIOUS TEMPERATURES ON HSP90 GENE EXPRESSION IN *P. CITRI* ADULT FEMALES

After the adult female mites were treated with abamectin solutions at concentrations equivalent to the LC_{25}, LC_{50} and LC_{75}, subgroups of these mites were further subjected to one of three temperatures: 18 °C, 25 °C or 32 °C. Surviving mites were collected after 1, 6, 12, 18, 24, 36 and 48 h. The untreated control group was kept at room temperature (25 °C). To determine the effect of temperature on the relative expression of the HSP90 gene from *P. citri*, the mites were treated for various times with one of 2 temperatures: 18 °C or 32 °C. The surviving adults were collected and placed into 1.5-mL centrifuge tubes. The specimens were frozen in liquid nitrogen and stored at -80 °C until needed in various analyses.

The RNA and cDNA were extracted based on the method described in section 1.2.2. The upstream and downstream primers were designed with Primer Premier 5.0 to detect the relative expression of HSP90 mRNA from *P. citri*, i.e., HSP90-F: 5'-TGAAAGTGATCCGCAAGAACC-3' andHSP90-R: 5'-CTGTCCTCGTGAATTCCAAG-3'. The upstream and downstream primers for the reference gene ELF1A (Niu et al. 2012) were as follows: ELF1A-F: 5'-GGCACTTCGTCTTCCACTTC-3', and ELFA-R: 5'-ATGATTCGTGGTGCATCTCA-3'.

The comparative C_T method (Livak & Schmittgen 2001) was used to detect the variation in the relative expression of the HSP90 gene in *P. citri* adult females at the 3 temperatures and various abamectin concentrations. Real-time fluorescent quantitative PCR (RT-qPCR) was conducted with SYBR Premix Ex Taq (TaKaRa). The PCR reaction system (20 μL) was as follows: SYBR Premix Ex TaqII, 10 μL; upstream and downstream primers, 0.5 μL each; cDNA template, 2.0 μL; ddH_2O 7.0 μL. The reaction conditions were as follows: predegeneration at 95 °C for 30 s; 95 °C for 5 s, 60 °C for 30 s, 40 cycles; 95 °C for 15 s, 60 °C for 15 s. The melting curve was recorded at 95 °C for 15 s. Each test had 3 replicates. The RT-PCR was conducted with Bio-Rad iQ5. At the end of the reaction, C_T values were collected and the relative gene expression was calculated with the $2^{-\Delta\Delta CT}$ method.

Reference genes are considered genes that maintain stable expression levels under different experimental conditions and in various tissues and cells. Although the relative HSP90 gene expression by the adult female mites changed with temperature and abamectin concentration, the data could be normalized through corrections using the expressed amount of reference genes in the corresponding sample.

RNA INTERFERENCE OF THE HSP90 GENE FROM *P. CITRI*

The double-stranded RNA (dsRNA) synthesized by Wuhan Cell Marker Biotechnology Co., Ltd. (5'-GCTAGGTTTGGGTATTGAT-3') was diluted (0.1 μg/μL, 0.2 μg/μL and 0.4 μg/μL) and then used to feed adult female mites that were at the same developmental stage. The dsRNA solution was applied under a microscope to the mouth parts of the mites by a clean fine brush (number 0) that had been treated with DEPC (diethyl pyrocarbonate) and dipped in the dsRNA solution. The mites in the control group were supplied with clean water. The adults were placed into an illumination incubator (25 ± 1 °C, RH 70–80%), and the surviving individuals were collected after 6, 12, 24 and 48 h and placed into 1.5 mL centrifuge tubes. After freezing in liquid nitrogen, the specimens were stored at -80 °C.

DATA PROCESSING

The mortality rates were corrected by Abbott's formula, and the data were used to fit a toxicity regression equation using SPSS 17.0. A χ^2 test was used to test the LC_{25}, LC_{50} and LC_{75} values at the 95% confidence interval to verify the accuracy of the toxicity regression equation using SPSS 17.0 software. The $2^{-\Delta\Delta CT}$ method was applied to calculate the relative expression of HSP90 mRNA in *P. citri* in the different treatment groups. The expression level in the control group was used as the background value. The difference in the relative expressions of HSP90 mRNA between treatment and control groups examined at the same temperature were analyzed with Student's *t*-test ($P < 0.05$). Differences across treatment groups were examined with multiple comparisons using One-Way ANOVA (LSD method) ($P < 0.05$).

Results and Analyses

CLONING AND SEQUENCE ANALYSIS OF FULL-LENGTH HSP90 GENE FROM *P. CITRI*

The 5'-end and 3'-end fragments were spliced with DNAMAN 5.0 after RACE amplification. This HSP90 gene consisted of 2,495 nucleotides with a complete open reading frame (ORF) of 2,169 nucleotides (GenBank: HQ257511.1), encoding a polypeptide of 721 amino acids with a predicted molecular weight of 83.44 kDa, a theoretical isoelectric point of 5.06, 3' untranslated region (UTR) of 228 bp, and 5' UTR of 98 bp (Fig. 1).

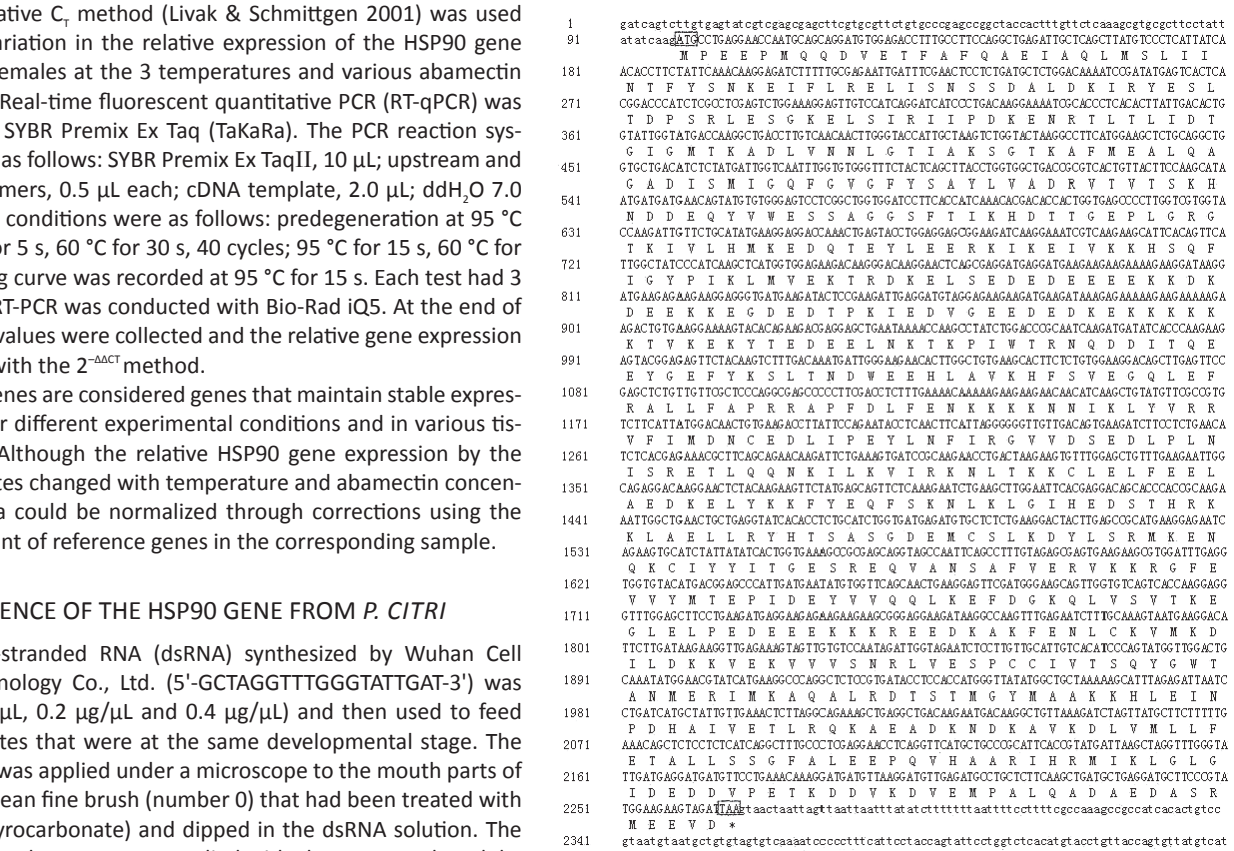

Fig. 1. The cDNA and deduced amino acid sequence of HSP90 of *Panonychus citri* adult females. The start and stop codons are boxed; the numbers on the left are for the positions of nucleotides and amino acids in the sequences.

Multiple alignments were performed with DNAMAN 5.0 on the amino acid sequences of HSP90 genes of *P. citri* McGregor and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), *Pteromalus puparum* (L.) (Hymenoptera: Pteromalidae), *Tetranychus cinabarinus* (Boisduval) (Trombidiformes: Tetranychidae). The results indicated that they shared several conserved motifs and displayed high amino acid sequence homology (Fig. 2).

ABAMECTIN TOXICITY BIOASSAY

In adult female *P. citri* the lethal concentrations of 1.8% abamectin EC were determines by a bioassay. Thus the LC_{25}, LC_{50} and LC_{75} were determined to be 0.0206 mg/L, 0.0572 mg/L and 0.1586 mg/L, respectively (Table 1).

EFFECTS OF ABAMECTIN CONCENTRATION AT 18 °C ON THE RELATIVE EXPRESSION OF HSP90 IN *P. CITRI*

After treatment at 18 °C with various concentrations of abamectin, the relative expression levels of HSP90 in *P. citri* increased within

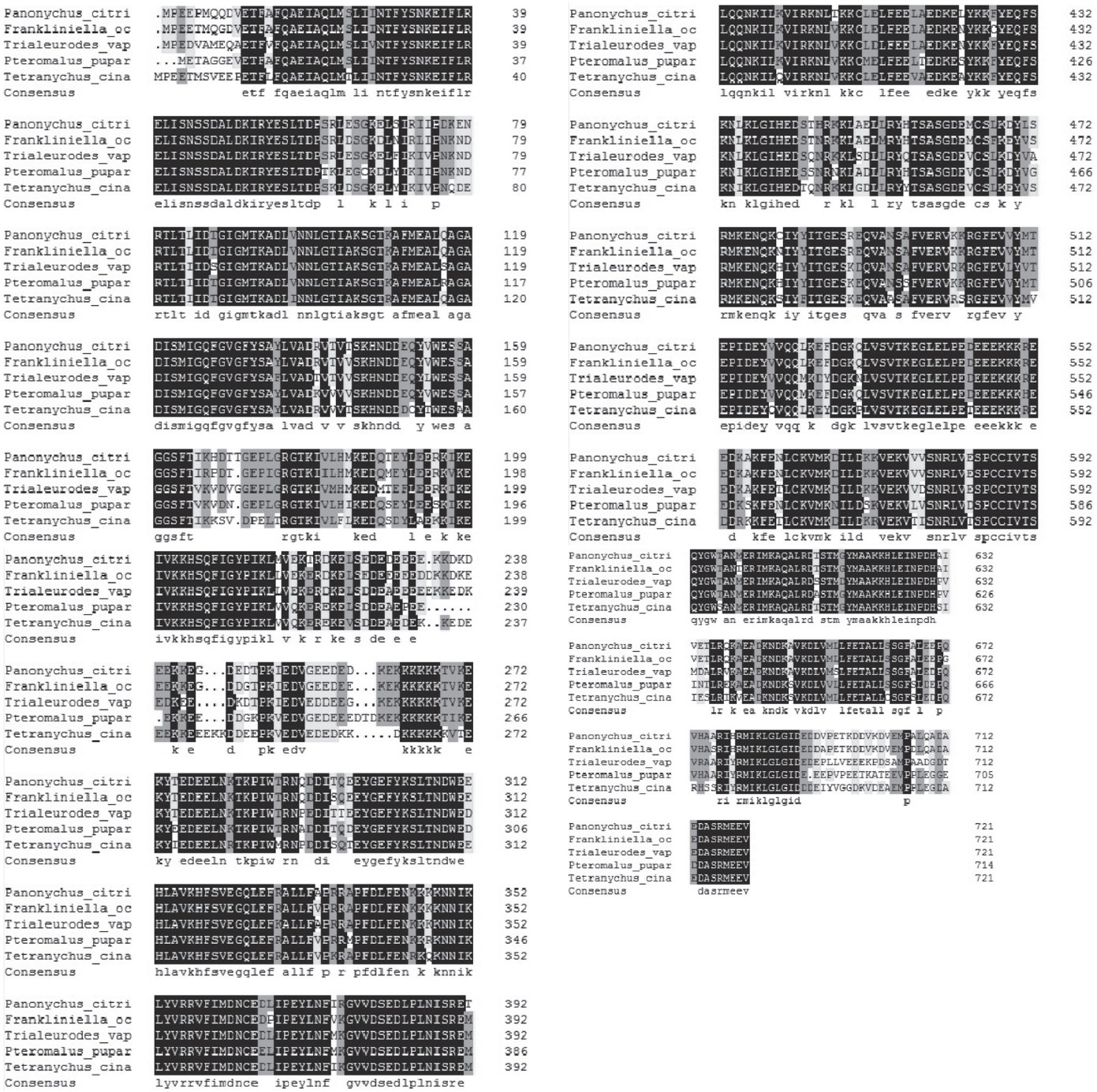

Fig. 2. Alignment of HSP90 amino acid sequence of *P. citri* McGregor and its homologus amino acid sequences from other species (*Frankliniella occidentalis*, *Trialeurodes vaporariorum*, *Pteromalus puparum*, *Tetranychus cinabarinus*).

Table 1. Toxicity of abamectin to *Panonychus citri* adult females.

Toxicity regression equation	R	χ^2	LC_{25} (CI_{95})* (mg/L)	LC_{50} (CI_{95}) (mg/L)	LC_{75} (CI_{95}) (mg/L)
Y = 6.8926 + 1.5233X	0.9987	0.5075	0.0206 (0.0148–0.0269)	0.0572 (0.0464–0.0686)	0.1586 (0.1339–0.1906)

*CI_{95}, 95% confidence interval.

a short time, but then gradually decreased to levels comparable with those in controls (Fig. 3).

EFFECTS OF ABAMECTIN CONCENTRATION AT 25°C ON THE RELATIVE EXPRESSION OF HSP90 IN *P. CITRI*

After treatment at 25 °C with various concentrations of abamectin, the relative expression levels of HSP90 in adult female *P. citri* decreased within a short time, then fluctuated, and subsequently were gradually restored to levels comparable with those in controls (Fig. 4).

EFFECTS OF ABAMECTIN CONCENTRATION AT 32 °C ON THE RELATIVE EXPRESSION OF HSP90 IN *P. CITRI*

With various concentrations of abamectin at 32 °C, the relative expression levels of HSP90 in adult female *P. citri* increased within a short time, then fluctuated, and subsequently were gradually restored to levels comparable with those in controls (Fig. 5).

EFFECTS OF TEMPERATURE ON THE RELATIVE EXPRESSION OF HSP90 GENE IN *P. CITRI*

The adult female mites were subjected to 1 of 2 temperatures (18 °C or 32 °C) for various lengths of time to determine the effects of temperature on the relative expression of HSP90 gene in *P. citri*. The control group was maintained at 25 °C. At 18 °C, the relative expression of HSP90 in *P. citri* increased within a short time, then fluctuated, and subsequently increased above the level of controls. At 32 °C, the relative expression of HSP90 in *P. citri* decreased within a short time, but then increased above the level of controls (Fig. 6).

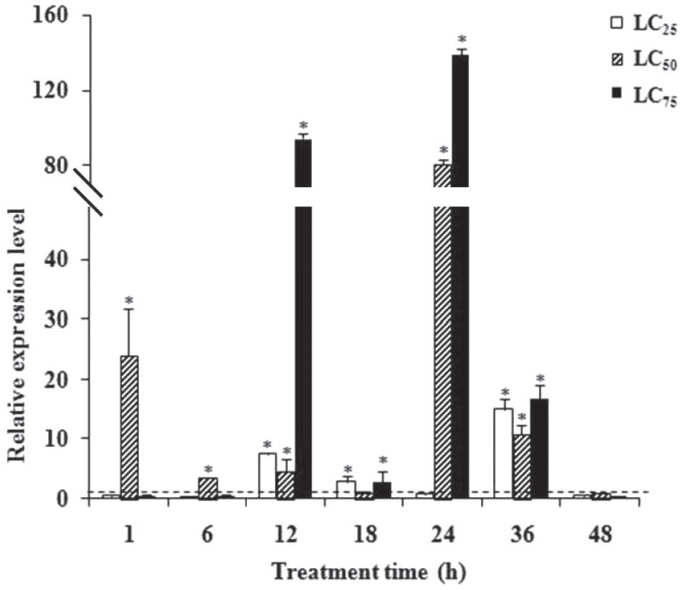

Fig. 4. Relative expressional levels of HSP90 mRNA in *P. citri* adult females after treatment at 25 °C with abamectin concentrations of LC_{25}, LC_{50} and LC_{75} for the indicated times. Data are shown as means of 3 replicates ± SD. The relative expression level of HSP90 mRNA in the control group is equal to 1.0 and is marked with a dashed line. The asterisk above the bar indicates a significance difference between treatment with abamectin and control group (*t*-test, $P < 0.05$).

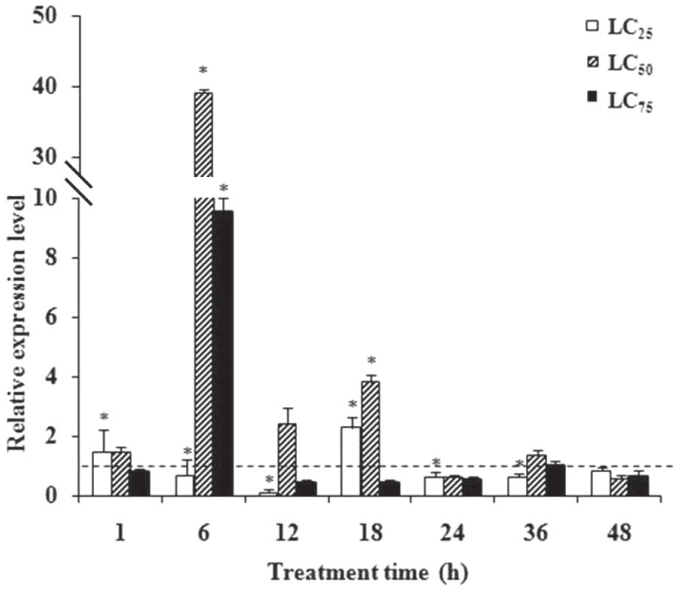

Fig. 3. Relative expressional levels of HSP90 mRNA in *P. citri* adult females after treatment with abamectin at LC_{25}, LC_{50} and LC_{75}levels for the indicated times at 18 °C.Data are shown as means of 3 replicates ± SD. The relative expression level of HSP90 mRNA in the control group is equal to 1.0 and is marked with a dashed line. The asterisk above the bar indicates a significance difference between treatment with abamectin and control group (*t*-test, $P < 0.05$).

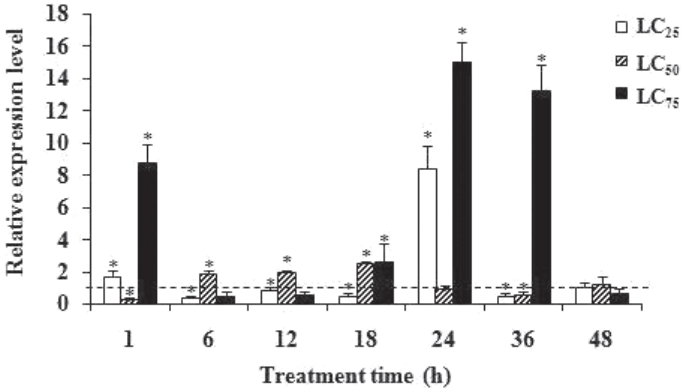

Fig. 5. Relative expressional levels of HSP90 mRNA in *P. citri* adult females after treatment at 32 °C with abamectin concentrations of LC_{25}, LC_{50} and LC_{75} for the indicated times. Data are shown as means of 3 replicates ± SD. The relative expression level of HSP90 mRNA in the control group is equal to 1.0 and is marked with a dashed line. The asterisk above the bar indicates a significance difference between treatment with abamectin and control group (*t*-test, $P < 0.05$).

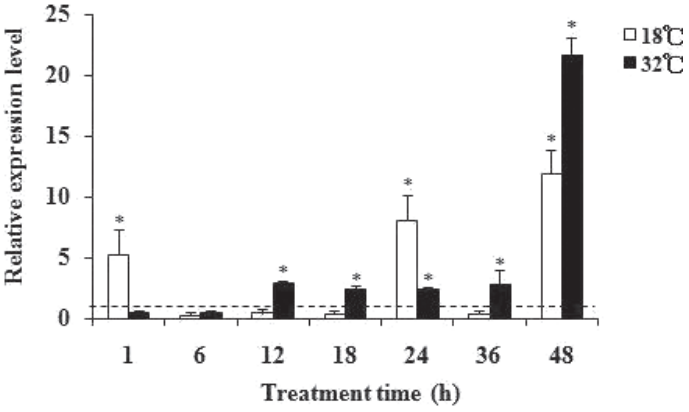

Fig. 6. Relative expressional levels of HSP90 mRNA in *P. citri* adult females subjected to 18 °C or 32 °C for the length of time indicated. Data are shown as means of 3 replicates ± SD. The relative expression level of HSP90 mRNA in the control group is equal to 1.0 and is marked with a dashed line. The asterisk above the bar indicates a significance difference between treatment with temperature and control group (*t*-test, $P < 0.05$).

DSRNA INTERFERENCE OF HSP90 IN *P. CITRI*

After the treatment with 0.1 µg/µL of dsRNA for 48 h, the relative expression levels of HSP90 in adult female mites were significantly down-regulated compared with those in the control group. With 0.2 µg/µL of dsRNA, the relative expression of HSP90 in *P. citri* first decreased but then increased 5.25 fold after 48 h ($P < 0.05$). After treatment with 0.4 µg/µL of dsRNA, the relative expression of HSP90 in *P. citri* fluctuated and then was gradually restored to levels comparable with those of controls (Fig. 7).

Discussion

Development of resistance in arthropods is mainly caused by decreased target sensitivity and increased activity of detoxifying enzymes (Ran et al. 2009; Liu et al. 2011). Although acaricide resistance in *P. citri* is rampant, there is less research on the elucidation of resistance mechanisms in this mite than on other arthropods, and, thus, the molecular mechanism for resistance of *P. citri* to most acaricides remains unclear (Chen et al. 2009; Niu et al. 2012). A previous study suggested that abamectin resistance of *T. cinnabarinus*is was related to an increase in its HSP90 expression (Zhu et al. 2013). In the present study, the full-length sequence of the HSP90 gene from *P. citri* was obtained by cloning, and then the effects of various abamectin concentrations on the HSP90 expression in *P. citri* adult females were analyzed. The results indicated that abamectin treatment at various times induced a marked up-regulation of the HSP90 gene in female adult *P. citri*, suggesting that the resistance of *P. citri* to abamectin may be related to the up-regulation of HSP90 gene.

As a chaperone protein, HSP90 maintains protein stability when stress tends to denature proteins and cause them to aggregate into nonfunctional structures. Thus under high or low temperature stress, HSP90 is overexpressed to maintain the stability of proteins in organisms, and when appropriate environmental conditions are restored, HSP90 expression returns to normal levels. This regulation is likely the reason why HSP90 was temporarily upregulated, as shown in Figs. 3–5, in the present study. The optimal temperature for normal growth of *P. citri* is 25 °C, probably because this temperature is optimal for enzyme activity. Either higher or lower temperatures may influence the regulatory element of the HSP90 gene. Therefore, as shown in Fig. 4, the variation of HSP90 expression in the adult female mites was more robust below 25 °C. Moreover, at the three temperatures, the mortality of the adult female mites increased with the pesticide concentration over time. When the effect of temperature on HSP90 gene expression in *P. citri* was further analyzed, we found that high temperature also induced an increase in the expression of this gene, suggesting that upregulating the HSP90 gene may also promote resistance to high temperatures in *P. citri*, but these results also indicated the need for further functional analysis.

RNA interference is an important tool for analyzing gene functions (Campbell et al. 2010). Two common methods used for RNA interference in arthropods are injection and feeding (Blandin et al. 2002; Soares et al. 2005; Turner et al. 2006; Walshe et al. 2009; Huvenne & Smagghe 2010). Because of the small size of mites, the injection method affords better results than feeding for gene silencing, but mortality is higher with the injection method. By contrast, the feeding method affords a lower mortality rate, but the gene silencing is not ideal (Chen et al. 2010; Liu et al. 2010). There are no previous reports of applying RNAi technology in *P. citri*. In the present study, the *P. citri mites* were fed dsRNA (0.1 µg/µL, 0.2 µg/µL and 0.4 µg/µL). The results indicated that this method effectively decreased HSP90 gene expression in *P. citri*; however, the decrease was unstable. This instability may have been caused by the feeding pattern of *P. citri* and the short duration of feeding on dsRNA. Complementary features of other molecular chaperone proteins might have limited the mortality rates observed in *P. citri* adult females caused by the various concentrations of the dsRNA. Additional systematic studies will be required to perfect the RNAi methodology for use in *P. citri*.

Among the current methodologies for functional gene analysis (Cerutti et al. 2011), RNA interference is appropriate for use in *P. citri*. However, the most effective dsRNA concentrations and lengths remain to be determined. In future studies, abamectin-resistant strains will be crossed and selected in an indoor laboratory. The RNAi methodology for functional HSP90 gene analysis will be established, and the difference in the expression of the HSP90 gene in acaricide-resistant and acaricide-sensitive *P. citri* strains at different temperatures will be

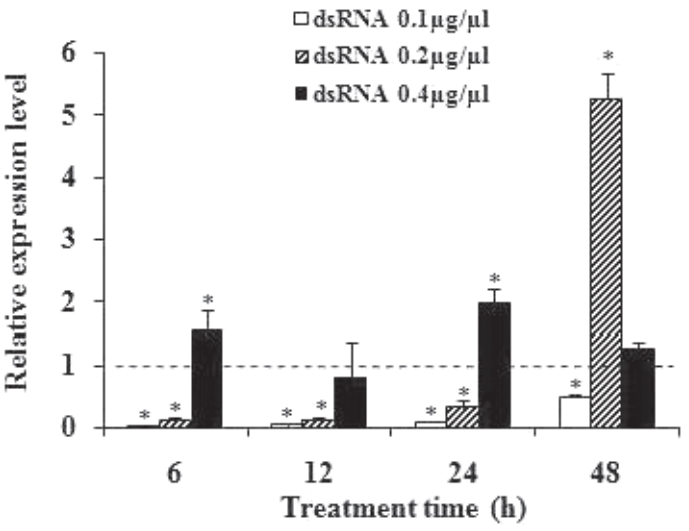

Fig. 7. Relative expressional levels of HSP90 mRNA in adult female *P. citri* McGregor after treatment with dsRNA at concentrations equivalent to 0.1 µg/µL, 0.2 µg/µL and 0.4 µg/µL. Data are shown as means of 3 replicates ± SD. The relative expression level of HSP90 mRNA in the control group is equal to 1.0 and is marked with a dashed line. The asterisk above the bar indicates a significance difference between treatment with dsRNA and control group (*t*-test, $P < 0.05$).

compared. The RNAi technology will be applied to analyze both the difference in survival of *P. citri* at high temperatures and the variation in sensitivity of abamectin-resistant strains after silencing of the HSP90 gene. Thus, the results in the present study that examined the effects of the HSP90 gene on the resistance of *P. citri* to high temperatures and chemicals may be confirmed. In summary, the results of the present study provide the molecular basis for future research on analyzing the relationship between the HSP90 gene and resistance of *P. citri* to high temperatures and abamectin.

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