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Corresponding authors:
Xiaoning Liu;
Email: liuxn0103@sina.com
Hongsheng Pan;
Email: panhongsheng0715@163.com

Functional analysis of *AgJHAMT* gene related to developmental period in *Aphis gossypii* Glover

Lianjun Zhang[1], Yuan Li[1], Xinhui Xu[1], Mengmeng Feng[1], Rukiya Turak[1], Xiaoning Liu[1] and Hongsheng Pan[2]

[1]Xinjiang Key Laboratory of Biological Resources and Genetic Engineering/National Demonstration Center for Experimental Biology Education, College of Life Science and Technology, Xinjiang University, Urumqi 830017, China and [2]National Plant Protection Scientific Observation and Experiment Station of Korla, Institute of Plant Protection, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, China

Abstract

Aphis gossypii is one of the most economically important agricultural pests that cause serious crop losses worldwide, and the indiscriminate chemical application causes resistance development in *A. gossypii,* a major obstacle to successful control. In this study, we selected the up-regulated expression gene *AgJHAMT*, which was enriched into juvenile hormone pathway though transcriptome sequencing analysis of the cotton aphids that fed on transgenic cotton lines expressing ds*AgCYP6CY3* (the TG cotton). The *AgJHAMT* gene was overexpressed in cotton aphids which fed on the TG cotton, and its expression profile during the nymphs was clarified. Then, silencing *AgJHAMT* could advance the developmental period of cotton aphids by 0.5 days compared with control groups. The T and t values of cotton aphids in the dsJHAMT treatment group (6.88 ± 0.15, 1.65 ± 0.06) were significantly shorter than that of the sprayed H_2O control group (7.6 ± 0.14, 1.97 ± 0.09) ($P < 0.05$), respectively. The fast growth caused by *AgJHAMT* silencing was rescued by applying the JH analogue, methoprene. Overall, these findings clarified the function of *AgJHAMT* in the developmental period of *A. gossypii*. This study contributes to further clarify the molecular mechanisms of delaying the growth and development of cotton aphids by the transgenic cotton lines expressing ds*AgCYP6CY3*.

Introduction

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is one of the most economically important pests throughout the world (Ebert and Cartwright, 1997) and is responsible for severe yield losses both through direct feeding and indirect virus transmission in various crops (Guncan *et al*., 2006; Wumuerhan *et al*., 2019; Zhang *et al*., 2020). Controlling cotton aphids in China is challenging due to its high reproductive capability, large population, tolerance and environmental adaptability (Eid *et al*., 2018). However, the indiscriminate and long-term chemical application poses an environmental risk and results in high levels of insecticidal resistance (Wu and Guo, 2005). In addition, widespread insecticide resistance in *A. gossypii* hinders chemical control (Zeng *et al*., 2021; Cheng *et al*., 2023). Therefore, new pest control strategies are urgently needed, such as targeting specific genes that can block pest development. This is important in contemporary pest management programmes to delay the development of insecticide resistance in cotton aphids.

RNAi has been considered a novel tool that promotes eco-friendly pest management strategy. Some researchers have investigated the mechanism of pest resistance to insecticides, including *Helicoverpa armigera*, *Ectropis oblique, and A. gossypii* using RNAi (Pan *et al*., 2020; Zheng *et al*., 2024). To explore novel control methods, some researchers had conducted studies on the growth and development of insects using RNAi to select appropriate target genes. Knockdown ferritin genes (*NlFer1* and *NlFer2*) led to retarded growth and 100% mortality in *Nilaparvata lugens* nymphs (Shen *et al*., 2021). Silencing *ApisCHS* led to mortality and moulting rate of *Acyrthosiphon pisum* was 44% and 51.3% after 72 h compared with ds*GFP* group, respectively (Ye *et al*., 2019). The knockdown of *CHS1* caused up to 43%, 47%, and 59% mortality in 3th instar *A. gossypii* after feeding dsCHS1 for 24, 48, and 72 h, respectively (Ullah *et al*., 2020). These studies suggested that insect growth and development genes can be used as target genes for RNAi to achieve effective pest control.

In insects, 20-hydroxyecdysone (20E) and juvenile hormone (JH) are the key hormones in regulating various development and reproductive processes (Jindra *et al*., 2013; Yamanaka *et al*., 2013). JH is one of the most critical sesquiterpenoid hormones, which plays various roles in the regulation of essential physiological processes, including moulting, metamorphosis, reproduction, diapause and migration (Riddiford *et al*., 2003; Zhao *et al*., 2017; Li *et al*., 2019; Xu *et al*., 2019; Riddiford, 2020; Oi *et al*., 2021; Zhang *et al*., 2022a). Some studies have revealed that juvenile hormone acid methyltransferase (*JHAMT*) is a rate-limiting enzyme in the JH synthesis pathway (Kinjoh *et al*., 2007; Marchal *et al*., 2011; Daimon and Shinoda, 2013; Cai *et al*., 2022). RNAi-mediated silencing of *JHAMT* in insects causes growth disorders, reduced reproductive quality, and diapause (Yin *et al*., 2020; Tian *et al*., 2021). In *Tribolium castaneum*, RNAi was performed on *TcJHAMT3* in 3rd instar larvae, causing early pupation and significantly smaller adults than that of the control group (Minakuchi *et al*., 2008). Furthermore, the mortality of *Leptinotarsa decemlineata* larvae fed ds*JHAMT1* and ds*JHAMT2* was 30.0% and 32.2%, respectively, while 66% and 62% of surviving larvae failed to pupate (Fu *et al*., 2016). Silencing the *JHAMT* gene decreased larval growth rate, higher larval mortality, pupation of fewer larvae and fewer adult emergence (Navale *et al*., 2017). These results suggested that *JHAMT* played an important role in insect growth and development. Our previous studies showed that feeding transgenic cotton lines expressing ds*AgCYP6CY3* (the TG cotton) not only increased the susceptibility of cotton aphids to neonicotinoid insecticides, but also delayed the development of cotton aphids (Zhang *et al*., 2022b).

To elucidate the molecular events underlying the physiological changes in cotton aphids that fed on the TG cotton, we selected the *JHAMT* gene that response to the TG cotton based on transcriptome sequencing analysis of cotton aphids in this study. Then the expression pattern of *AgJHAMT* during the nymph stages and after *AgCYP6CY3* gene silencing were detected, respectively. Subsequently, the gene function was analysed by spraying-mediated RNAi combined with the methoprene rescue experiment. This study laid a foundation for further investigation of the mechanism of the TG cotton delayed the development of cotton aphids and helped to evaluate its potential for developing novel control strategies against this pest.

Materials and methods

Insects

The susceptible cotton aphid's population was collected in 2010 from the Anningqu Town in Urumqi, Xinjiang province, China. They were reared on cotton seedlings (*Gossypium hirsutum*) under 25 ± 1 °C and relative humidity of 50–60% with a 16 h L: 8 h D photoperiod in the Xinjiang laboratory of biological resources and genetic engineering of Xinjiang University. The newborn nymphs (<12 h) were used in RNAi and methoprene rescue experiments.

Transcriptome sequencing (RNA-Seq)

The newborn nymphs were released on the non-transgenic cotton (the NT cotton) and the transgenic cotton lines expressing ds*AgCYP6CY3* (the TG cotton), respectively. The cotton seedlings were covered with plastic cups to prevent the aphids escaping. Each treatment had three biological replicates. Then the total RNA of cotton aphids that fed on the NT cotton and the TG cotton for 36 h were extracted and used to detect the relative expression of *CYP6CY3* in cotton aphids, transcriptome sequencing and transcriptome verification experiments. Total RNA was extracted using TransZol Up Plus RNA Kit (TransGen Biotech, Beijing, China) following the manufacturer's instructions. The quality and concentration of RNA were confirmed using agarose gel electrophoresis and NanoDrop-1000 Spectrophotometer (Thermo Scientific, CA, USA), respectively. Part of the total RNA was sent to Biomarker Technologies Co., Ltd. (Beijing, China) for library preparation. The other part was reverse transcribed into cDNA using TransScript All-in-one First-Strand cDNA Synthesis Supermix for qPCR (One-Step gDNA Removal) kit (TransGen Biotech, Beijing, China) following the manufacturer's instructions, and cDNA templates were stored at −80 °C for reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis to verify the reliability of transcriptome results.

Multiple sequence alignments and phylogenetic analysis

The *AgJHAMT* gene was cloned. Briefly, the PCR procedures were as follows: initial pre-denaturation at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min, and a final elongation step at 72 °C for 10 min. Target amplicons were purified, then products were transferred to vector pMD19-T (TakaRa, Dalian, China) for sequencing. The *AgJHAMT* sequence was analysed using Primer Premier 5 and DNAMAN. Other JHAMT protein sequences were obtained from the National Center for Biotechnology Information (NCBI). The phylogenetic tree was constructed with the neighbour-joining method based on 1000 bootstrap replicates using MEGA 10.0. The primers used in this study were shown in table 1.

Expression pattern of AgJHAMT in A. gossypii

To study the effect of the TG cotton on the growth and development of cotton aphids, we detected the relative expression level of *AgJHAMT* in cotton aphids that fed on the TG cotton. In addition, we detected the expression pattern of *AgJHAMT* in cotton aphids that 8 hours before and 8 hours after each moulting peak as each instar's early and late stages, respectively. The total RNA and cDNA of *A. gossypii* were obtained according to the above method. RT-qPCR was performed on the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems, Foster city, CA, USA). The experiment was conducted with 3 independent biological replicates, each with 2 technical replicates. The relative expression level was calculated using the $2^{-\triangle\triangle Ct}$ method (Livak and Schmittgen, 2001), and the *18S* rRNA was used as the internal control.

Synthesis of double-stranded RNA (dsRNA)

We truncated the coding sequence of the *AgJHAMT* gene as the interference fragment, and synthesised dsRNA according to the MEGAscript™ RNAi kit (Ambion, Huntingdon, USA). The dsRNA was analysed by 1% agarose gel electrophoresis and quantified using NanoDrop-1000 spectrophotometer. Green fluorescent protein dsRNA was synthesised under identical conditions and was used as a control. The specific primers of two dsRNA fragments were shown in table 1.

RNA interference (RNAi)

The silencing efficiency of *AgJHAMT* and its effects on the growth and development of cotton aphids were investigated following our previous method (Wei *et al*., 2021). Synthetic ds*GFP* and ds*JHAMT* (the final concentration is 500 ng/μL), approximately 200 μL per plant were directly sprayed on the cotton seedlings containing the newborn nymphs using a 2 mL volume sprayer

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Table 1. Primer sequences

Primer name	Primer Sequence (5'-3')	Application
JHAMT F	CGGAATTCATGATTTGCCCAAAGCAG	CDS
JHAMT R	CCGCTCGAGTTAATCTTTGATAGCATGAACAG	CDS
JHAMT F	TGTGGACCAGGCGACATAAC	RT-qPCR
JHAMT R	AGAGCAATCATTGGCATTTTC	RT-qPCR
18S rRNA F	CCGGAAAGATTGACAGATTGAG	RT-qPCR
18S rRNA R	CAGGACAGAGTCTCGTTCGTTATC	RT-qPCR
dsJHAMT F	TAATACGACTCACTATAGGGAGAAAATGCCAATGATTGC	dsRNA synthesis
dsJHAMT R	TAATACGACTCACTATAGGGAGAATATACATTAAGGTTGTTCTTCC	dsRNA synthesis
dsGFP F	TAATACGACTCACTATAGGGAATACGTGCAGGAGAGGACC	dsRNA synthesis
dsGFP R	TAATACGACTCACTATAGGGATTCATCCATGCCATGTGTAATC	dsRNA synthesis

Note: The underlined sequence is the T7 promoter sequence added at the 5'end of the primer.

from four directions, respectively. The dsRNA was sprayed only once during the entire experiment. The cotton aphids fed on the leaves were directly exposed to dsRNA, and the aphids continuously fed on dsRNA-sprayed cottons. The plastic cups covered cotton seedlings to prevent aphids from escaping. The sprayed H_2O and dsGFP were used as control groups, respectively. Each treatment was repeated 3 times.

After RNAi, cotton aphids were collected at 2nd instar, 3rd instar, 4th instar and A^{1} (adult 1st day) from the sprayed H_2O, ds*GFP* control groups and the sprayed ds*JHAMT* treatment group to detect the relative expression level of *AgJHAMT* gene using RT-qPCR according to the above method.

After the newborn nymphs were treated with dsRNA, the number of moults, deaths and newborn progeny nymphs were recorded and then removed until all treated adult aphids died. The life table was constructed for aphids using data from the study described above. Life table parameter calculation formula: net reproductive rate: $R_0 = \Sigma(l_x m_x)$, the mean generation time: $T = \Sigma(x l_x m_x)/\Sigma(l_x m_x)$, the intrinsic rate of increase: $r_m = (\ln R_0)$ / T, finite rate of increase: $\lambda = \exp^{rm}$, population doubling time: $t_d = \ln 2/r_m = 0.6931/r_m$, x represents age in days, l_x represents the age-specific survival rate, m_x represents the age-specific fecundity, $l_x m_x$ represents age-specific maternity.

Rescue assay by methoprene (juvenile hormone analogue, JHA) treatment

A rescue assay was conducted to study the effects of methoprene on cotton aphids after *AgJHAMT* silencing. The newborn nymphs were sprayed with ds*JHAMT* for 1.5 days, then sprayed with methoprene (0.01 µg/µL). The experiment was conducted with three independent biological replicates.

Statistical analysis

Statistical analyses were performed using SPSS 20.0. Significant differences between the three groups were calculated using one-way analysis of variance (ANOVA) in conjunction with Tukey's test, and different letters were used to indicate significance at $P < 0.05$, while Student's t-test were used to analyse pairs of groups ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

Results

Screening AgJHAMT *by transcriptome sequencing analysis*

In the current study, high-quality base reads were obtained; all the reads and base counts and their qualities were listed in table S1. In total, 87.52 Gb of clean data were obtained. The sequencing data had an average GC content of 38.34% and >94.80% of each sample had a quality score of Q30. The efficiency of comparison between reads and reference genomes ranged from 90.13% to 91.33%. We selected 15 differentially expressed genes for RT-qPCR and compared them with transcriptome sequencing analysis, indicating that the RNA-Seq results were reliable (fig. S1). The primers used for verification were listed in table S2.

Compared with the cotton aphids that fed on the NT cotton, 151 differentially expressed unigenes (DEGs) were found in the cotton aphids fed on the TG cotton, which included 51 up-regulated genes and 100 down-regulated genes (fig. 1a). In the KEGG analysis, 151 DEGs were assigned to 47 KEGG pathways, and the top 20 significantly enriched KEGG pathways were shown in fig. 1b. Of these, 13 were involved in nutrient metabolism, such as sugar metabolism (4), insect hormone biosynthesis (1), amino acid related metabolism (1), and lipid metabolism (7). Three pathways were related to metabolic detoxification: drug metabolism-cytochrome P450, metabolism of xenobiotics by cytochrome P450 and lysosome. According to the GO terms, DEGs were divided into three categories (biological processes, molecular functions, and cellular components) containing 42 variety classes. Catalytic activity (50), binding (38), and single-organism processes (40) contained the most UniGenes in the three categories (fig. 1c). According to the above transcriptome data analysis results, we found *JHAMT* involved in insect hormone biosynthesis was up-regulated expression in cotton aphids which fed on the TG cotton, and it was selected to explore its function in *A. gossypii.*

Cloning and sequence analysis of AgJHAMT

Using cDNA from adult cotton aphids as template, an 801 bp ORF sequence of a *JHAMT* orthologue (*AgJHAMT*) was amplified by PCR and then sequenced. A comparison of the amino acid sequences of five JHAMTs indicated that the putative SAM-binding motif is well conserved in all methyltransferases

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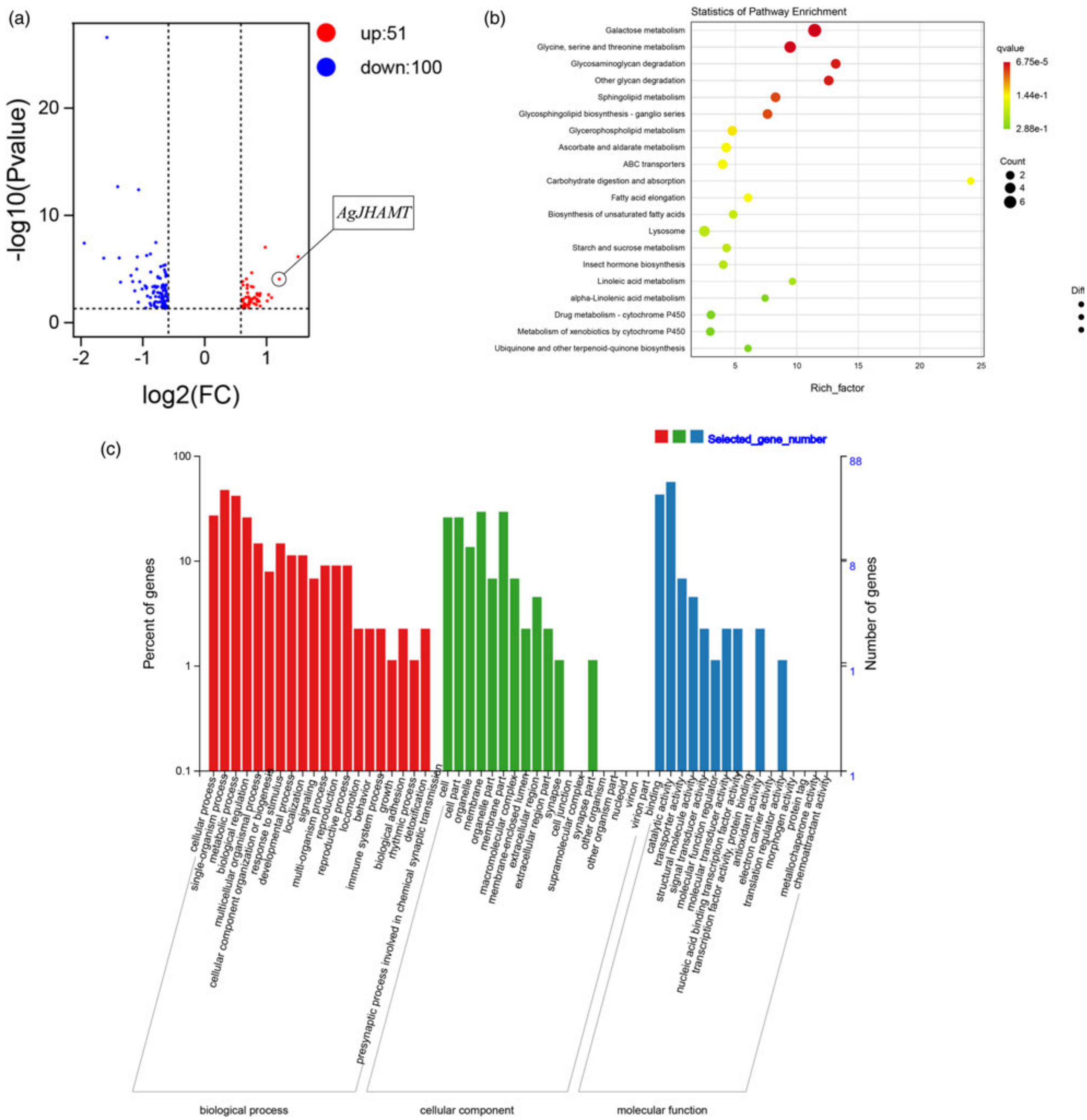

Figure 1. Function annotation and enrichment of DEGs. (a) volcano plot of differentially expressed genes of *A. gossypii* fed on the TG cotton (red spots represent significantly up-regulated genes; blue spots represent significantly down-regulated genes). (b) the most enriched KECG pathways of *A. gossypii* after fed on the TG cotton. (c) GO function annotation analysis of *A. gossypii* which fed on the TG cotton.

(fig. 2). Then the phylogenetic tree was constructed with JHAMT from *A. gossypii* and other insect species by MEGA 10.0. It clustered the AgJHAMT protein in a well-supported Hemiptera clade (fig. 3). This result demonstrated that *AgJHAMT* gene had been cloned and it was closely related to the JHAMT of other Hemiptera.

Analysis of the expression pattern of AgJHAMT

In addition, we investigated the expression pattern of *AgJHAMT* in cotton aphids that fed on the TG cotton. The results showed that the relative expression level of *AgJHAMT* in the TG group was significantly higher than that of the NT group at 24 h, 48 h, 72 h and 96 h, respectively. It was twice as high as that of the NT group at 96 h (fig. 4). This result suggested that a high expression level of *AgJHAMT* of cotton aphids that fed on the TG cotton might lead to its developmental delay.

The temporal expression profile of *AgJHAMT* was examined using RT-qPCR analysis. The results showed that *AgJHAMT* was expressed during the nymph stages of cotton aphids, and its relative expression increased with development. The relative expression level in the early stages of each instar was significantly higher than that of the corresponding late stages (fig. 5). This result showed that the expression level of *AgJHAMT* fluctuated

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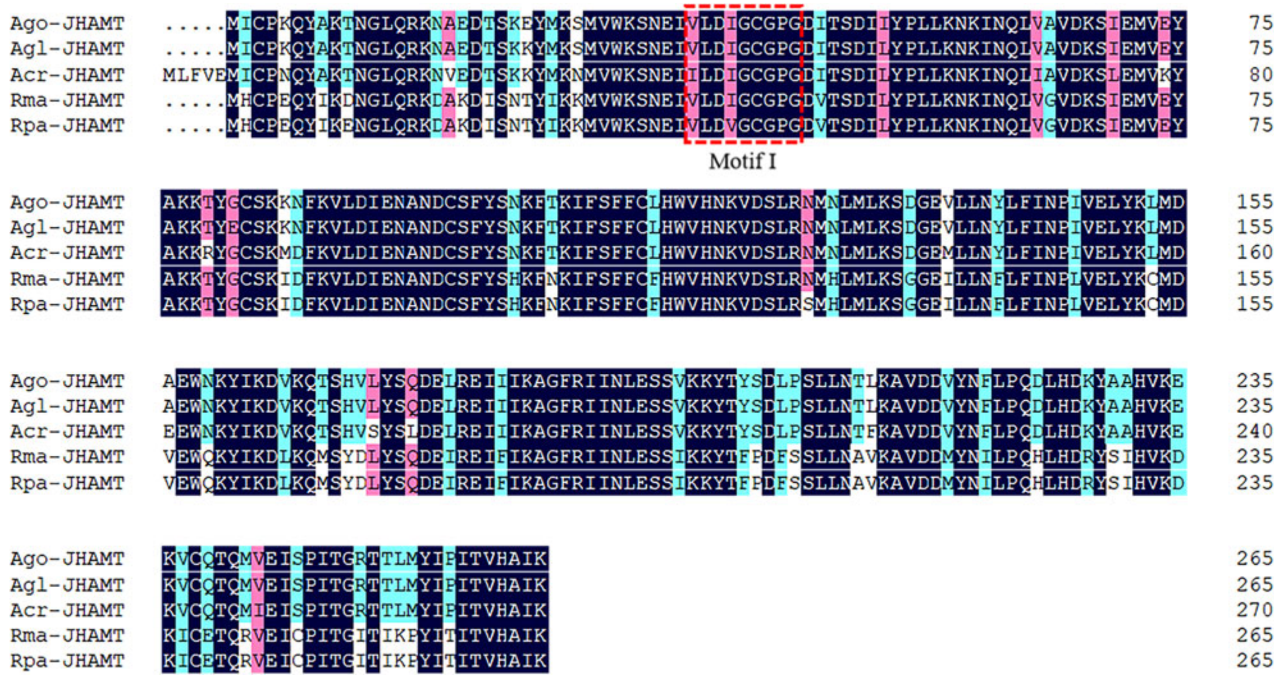

Figure 2. Multiple alignments of amino acid sequences of JHAMT in four insect species. Identical residues are indicated with black backgrounds; high homology residues are indicated with blue backgrounds. The red dotted box represents the SAM-binding motif. The details and GenBank accession numbers of the six JHAMTs are listed in the order illustrated: *A. gossypii* JHAMT (XP_027843037.2); *Aphis glycines* JHAMT (KAE9531301.1); *Aphis craccivora* JHAMT (KAF0764091.1); *Rhopalosiphum maidis* JHAMT (XP_026813805.1); *and Rhopalosiphum padi* JHAMT (WJN62156.1).

with instars, implying that the function of this gene was related to the developmental period of *A. gossypii*.

Effect of silencing AgJHAMT *on the growth and development of* A. gossypii

Cotton aphids were collected at 2[nd] instar, 3[rd] instar, 4[th] instar and A[1] from control and treatment groups to detect the silencing efficiency of target gene (fig. 6). The result showed that the relative expression level of *AgJHAMT* was no significance between the sprayed H_2O and ds*GFP* control groups. With the development of the aphids, the target gene expression level was significantly lower in the ds*JHAMT*-treatment group than that of the sprayed H_2O and ds*GFP* control groups ($P < 0.05$), respectively. The relative expression level of *AgJHAMT* decreased by 66.2%, 42.9%, 67.8% and 43.6% in the 2[nd] instar, 3[rd] instar, 4[th] instar nymph and A[1] compared with the sprayed H_2O control group, respectively, indicating that the expression level was effectively silenced by spraying ds*JHAMT*.

To further explore the role of *AgJHAMT* in the growth and development of cotton aphids, the newborn nymphs were sprayed with synthetic ds*JHAMT*. The result showed that there were four

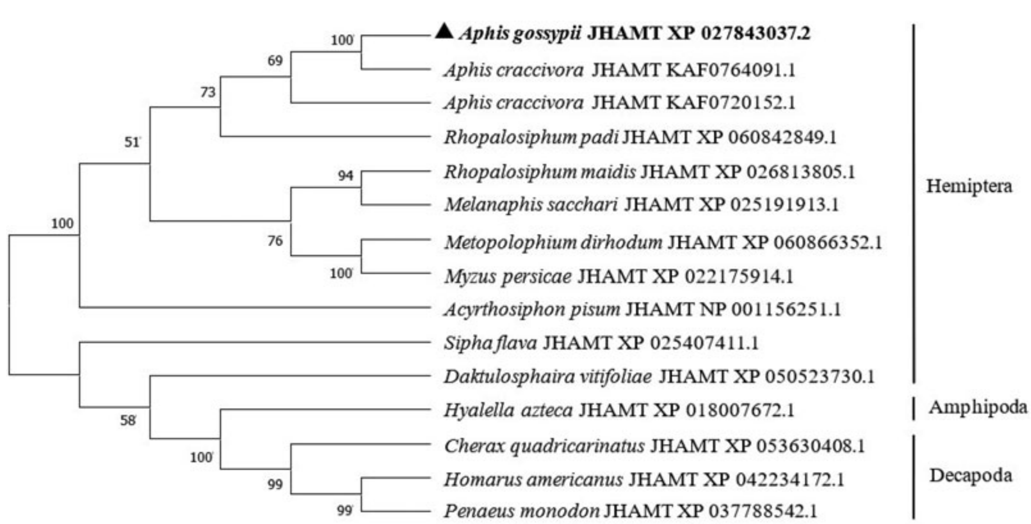

Figure 3. Phylogenetic analyses of AgJHAMT. The phylogenetic tree is based on amino acid sequences using the neighbor-joining method with a bootstrap of 1000 through MEGA 10.0. The numbers at the branches' nodes represent the bootstrap support level for each branch.

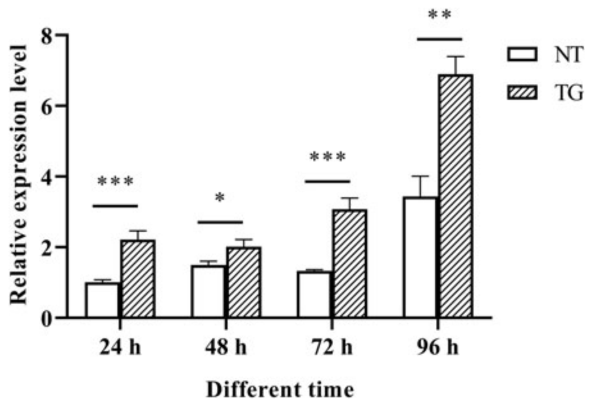

Figure 4. The relative expression level of *AgJHAMT* in *A. gossypii* which fed on the TG cotton. NT: *A. gossypii* which fed on the NT cotton; TG: *A. gossypii* which fed on the TG cotton. * Indicates a significant difference between the NT group and the TG group (mean ± SE, n = 3, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test).

peaks in the frequency distribution of the number of nymphs moulting, which corresponded to the four developmental stages of cotton aphids (fig. 7). The generation duration of cotton aphids treated with ds*JHAMT* was 4 days, while 4.5 days durations were observed for the sprayed H_2O and ds*GFP* control groups. The overall developmental period of cotton aphids in the treatment group was 0.5 days earlier than that of the two control groups. This result suggested that the developmental period of cotton aphids was advanced after *AgJHAMT* silencing.

Mortality and reproduction hadbeen recorded daily intervals to assess the population dynamics of cotton aphids following the application of ds*JHAMT*. There had a weak effect on the death and fecundity of cotton aphids (fig. S2). Based on the above data, the effects of *AgJHAMT* silencing on the growth and development of cotton aphids were further investigated. We constructed a life table to evaluate various population parameters of cotton aphids after spraying ds*JHAMT* (table 2). The results showed that the T and t values of cotton aphids in ds*JHAMT* treatment group (6.88 ± 0.15) (1.65 ± 0.06) were significantly shorter than that of the sprayed H_2O control group (7.6 ± 0.14) (1.97 ± 0.09) ($P < 0.05$), respectively. These results suggested that silencing the *AgJHAMT* gene shortened the mean generation time and population doubling time of cotton aphids, disrupted its growth and development.

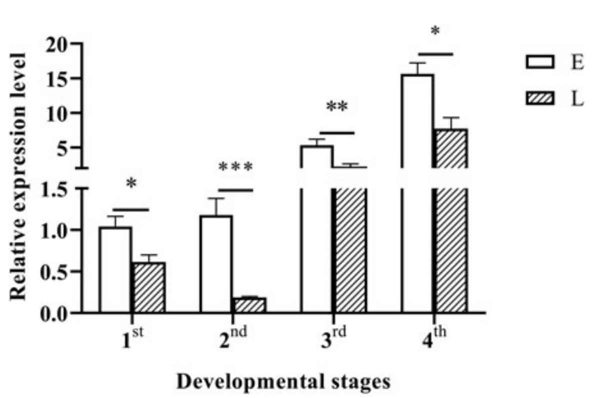

Figure 5. The relative expression level of *AgJHAMT* in different developmental stages of *A. gossypii*. E: early stage of nymphs; L: late stage of nymphs. * Indicates a significant difference between the early stage and the late stage (mean ± SE, $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, Student's *t*-test).

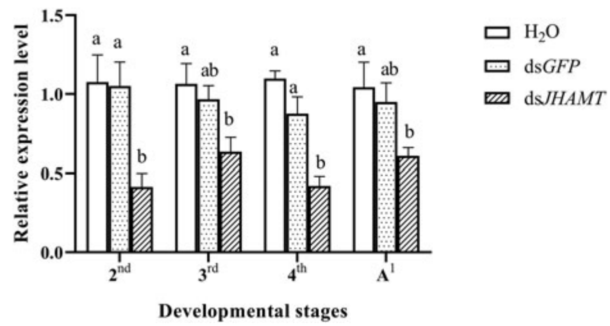

Figure 6. The relative expression level of *AgJHAMT* in *A. gossypii*. Different letters indicate statistically significant differences (mean ± SE, $n = 3$, $P < 0.05$, Tukey's HSD test).

*Methoprene (juvenile hormone analogues, JHA) rescues the effect of ds*JHAMT

The aphids growth were significantly delayed following RNAi-mediated silencing the *AgJHAMT*. A rescue assay with methoprene was performed to investigate whether the lack of JH caused this result. The developmental period of aphids was recorded. The four moulting times of cotton aphids in ds*JHAMT* treatment group were shorter than those of the H_2O control group. In the JHA rescue group, the first moulting time (before rescue) of nymphs treated with ds*JHAMT* was 0.5 day earlier than that of the sprayed H_2O control group, and then one of the two groups that sprayed ds*JHAMT* was sprayed JHA (0.01 μg/μL) to rescue. The second moulting time (the 1st moulting time after rescue) of nymphs was still 0.5 day earlier than that of the sprayed H_2O control group. But the third moulting time (the 2nd moulting time after rescue) of nymphs was longer than that of the ds*JHAMT* group, which was coinciding with the 3rd moulting time from the H_2O control group, and then the next moulting time of nymphs was the same as the 4th moulting time from the H_2O control group (fig. 8a). These above results suggested that methoprene (JHA) could do rescue the rapidly developmental period of cotton aphids caused by *AgJHAMT* silencing.

In addition, the age-specific survival rate (l_x) result showed that both the l_x curve of cotton aphids in the sprayed ds*JHAMT* and JHA rescue treatment groups had a tendency to decrease compared with that of the H_2O control group, respectively (fig. 8b). And the life cycle of the cotton aphids in the H_2O, ds*JHAMT* and JHA rescue treatment groups was 25, 20, and 24 days, respectively. The life cycle of the cotton aphids in the sprayed ds*JHAMT* treatment group was 5 days earlier than that of the sprayed H_2O control group. The rescued group using methoprene was similar to the sprayed H_2O control group. These results indicated that the life cycle of cotton aphids was advanced by silencing *AgJHAMT*, which was rescued though treatment with JHA.

Discussion

Transcriptome sequencing is used to identify the key genes and pathways linked with the growth and development of the insect pest. For example, cheng *et al.*, used transcriptome sequencing to screen the *HNF* gene affecting embryonic development and egg hatching in *N. lugens* (Cheng *et al.*, 2020). The transcriptome sequencing was used to select and knock out the gene *EcRA* associated with insect moulting, which affects *Spodoptera exiguais*

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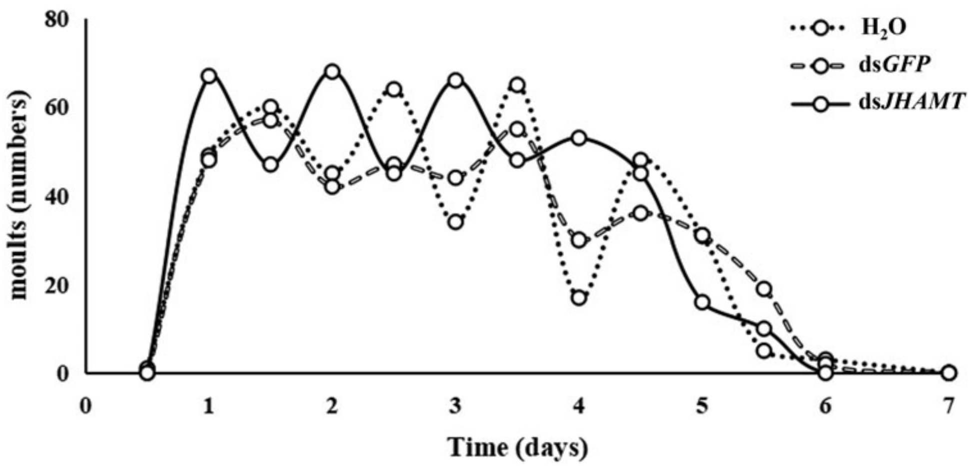

Figure 7. Developmental period of *A. gossypii* sprayed with ds*JHAMT*.

mortality and the ecdysone signalling pathway (Zhang *et al.*, 2021). In insects, ecdysteroids and sesquiterpenoid hormones of arthropods play vital roles in regulating various developmental processes such as moulting, growth, and metamorphosis (Daimon *et al.*, 2012; Yamanaka *et al.*, 2013; Mirth *et al.*, 2014). The titres of these two hormones are precisely coordinated by biosynthesis and metabolism pathways to regulate the physiological and developmental processes. Although ecdysteroids initiate the moulting process, JH determines the nature of the moulting (Lenaerts *et al.*, 2016).

As an important gene in insect JH biosynthesis, *JHAMT* affects the physiological processes of insect growth, development and reproduction (Navale *et al.*, 2017; Zhou *et al.*, 2022). *JHAMT* was characterised in several insect species, including *Holcocerus hippophaecolus* (Zhang *et al.*, 2016) and *T. castaneum* (Xu *et al.*, 2022), closely related to their growth and development. In addition, RNAi-mediated silencing of *JHAMT* in *Bactrocera dorsalis* greatly decreased the JH III titre, affecting the body length and overall size of larvae (Zhou *et al.*, 2022). The *H. armigera* pupation was reduced following the silencing of *JHAMT* (Jaiwal *et al.*, 2020). The expression changes of *JHAMT* affects the JH titre, thus disrupting the growth and development process of insects. Other studies had shown that the developmental expression profile of *JHAMT* in *Drosophila melanogaster* correlates with changes of the JH titre (Niwa *et al.*, 2008). The research showed that the content of JH titre increased in the early 4th instar, while decreasing in the later age of the 4th instar. The JH titre was sharply increased in the early 5th instar. Similarly, the content of *JHAMT* gene was also reduced in larvae (Kinjoh *et al.*, 2007). Our study found that the relative expression of *AgJHAMT* in the early stages of each instar was significantly higher than that of the corresponding late stages. Therefore, we speculated that the fluctuation of *AgJHAMT* expression with instar maybe due to the influence of JH titres. It also implied that *AgJHAMT* was closely related to the developmental period of *A. gossypii*.

Generally, functional genes involved in insect development or key metabolic processes could be suitable for RNAi targets (Kola *et al.*, 2015; Yu *et al.*, 2016). *JHAMT* is a specific target for developing new insect growth regulators or insecticides because it regulates JH synthesis in insect development and reproduction (Hiruma and Kaneko, 2013). Studies have shown that the body length and the overall size of *B. dorsalis* larvae after silencing *JHAMT* were significantly decreased and reduced (Zhou *et al.*, 2022). Other studies have shown that compared with the dsGFP control, the lower level of *JHAMT1* expression leads to reproductive arrest of cabbage beetles (Tian *et al.*, 2021). In this study, transcriptome data analysis results showed that *JHAMT* was up-regulated in the insect hormone metabolic pathway directly related to the growth and development of cotton aphids. The study also showed that overexpression of *AgJHAMT* gene in cotton aphids after fed on the TG cotton (fig. 2). These results indicated that the developmental retardation of cotton aphids by the TG cotton might be contributing to the up-regulation of *AgJHAMT*. Additionally, the developmental period of cotton aphids was advanced after RNAi-mediated silencing of *AgJHAMT*, compared with the sprayed H_2O control group, r_m and λ of cotton aphids were also significantly increased, T and t were significantly decreased. These results further clarify our previous research (Zhang *et al.*, 2022b). However, the influence of cumulative mortality and cumulative reproduction were subtle in the ds*JHAMT* treatment group compared with the sprayed H_2O and ds*GFP* control groups, the results showed that the cumulative mortality was not significantly increased consistently by spraying ds*JHAMT*, but its cumulative reproductive was significantly increased from 5th to 9th days ($P < 0.05$) (Fig. S2). The reason for this result might be related to the concentration of juvenile hormone in *A. gossypii*. The appropriate concentration of hormones or analogues to regulate insect life activity is important, the effects caused by high or low concentrations of hormones are different or opposite (Staal, 1975, 1986; Champlin and Truman, 1998; Orth *et al.*, 1999). It is reported that different

Table 2. Life table parameters of cotton aphids sprayed with ds*JHAMT*

Population parameters	H_2O	ds*GFP*	ds*JHAMT*
R_0 (offspring/individual)	14.24 ± 1.86	18.89 ± 1.26	18.29 ± 0.93
r_m(d^{-1})	0.35 ± 0.02^{b}	0.38 ± 0.02^{ab}	0.42 ± 0.01^{a}
λ(d^{-1})	1.43 ± 0.02^{b}	1.46 ± 0.03^{ab}	1.53 ± 0.02^{a}
T(d)	7.6 ± 0.14^{a}	7.86 ± 0.32^{a}	6.88 ± 0.15^{b}
t(d)	1.97 ± 0.09^{a}	1.87 ± 0.12^{ab}	1.65 ± 0.06^{b}

Note: The data in the table are mean ± SE; different lowercase letters in the same row indicate that there is a significant difference at the 0.05 level between different treatments.

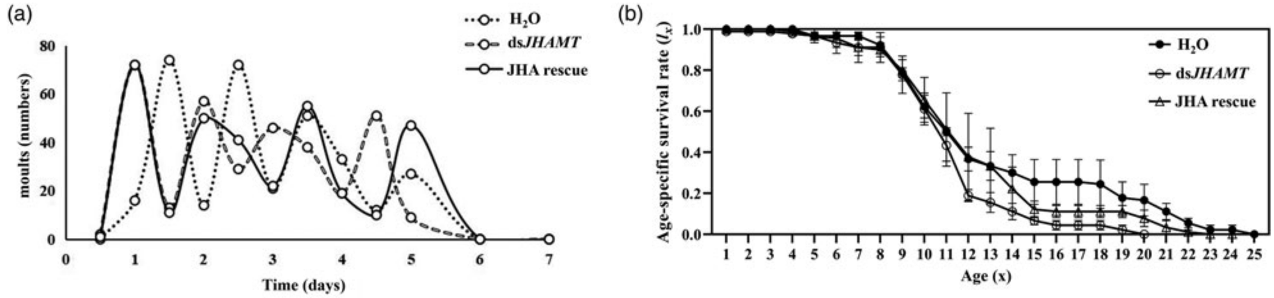

Figure 8. Effects of methoprene rescue on growth and development of *A. gossypii.* (a) Developmental period. (b) Age-specific survival rate (l_x).

concentrations of juvenile hormone analogues (0.1 μg/2μL, 1 μg/2μL, 5 μg/2μL, 10 μg/2μL) were applied to treat *H. armigera*, and the results showed that low concentrations of JHA had no significant effect on F1 generation survival of *H. armigera*, but the cumulative survival rate at 10 μg/2μL was 63% significantly lower than that of the control group. The total number of eggs laid female adult increased first, then decreased with JHA concentrations increasing. The results showed that in insect, the effects of JHA on different physiological processes had different threshold (Chen, 2013). Alternatively, JHAMT protein expression was significantly reduced by RNAi in *Drosophila melanogaster*, but there was no significant effect on its development (Niwa *et al.*, 2008). This may be related to individual differences, and different thresholds in different individuals (Tibbetts *et al.*, 2011). In addition, *A. gossypii* belongs to r-strategy insect which are achieved by a distinctive life-history strategy consisting of rapid development, early reproduction and a short life cycle when the aphids were treated with ds*JHAMT*. The rescue experiment showed that juvenile hormone analogues (methoprene) did rescue the rapid growth of *A. gossypii* caused by silencing *AgJHAMT*, and its whole life cycle was almost coinciding with that of the sprayed H_2O control group after methoprene rescue. Similar findings have been reported in other studies. For example, in *L. decemlineata*, feeding on ds*JHAMT1* and ds*JHAMT2* caused 2nd instar and 4th instar mortality, and the JH analogue pyriproxyfen rescued the negative performance (Fu *et al.*, 2016). Knockdown of *TcMT3* in *T. castaneum* larvae resulted in precocious larval-pupal metamorphosis, which was rescued by methoprene (Minakuchi *et al.*, 2008). These results indicated that *AgJHAMT* silencing promoted the development of cotton aphids, and illuminated the function of *AgJHAMT* in the developmental period of cotton aphids.

To sum up, we obtained the differentially expressed gene *AgJHAMT*, which was enriched in the hormone synthesis pathway related to the growth and development of cotton aphids by transcriptome analysis. These results of the *AgJHAMT* expression pattern, *AgJHAMT* silencing and JHA rescuing experiments elucidated that the *AgJHAMT* gene played an important role in the developmental period of nymphs. These results not only implied that low expression of *AgJHAMT* would accelerate development of cotton aphids to a certain extent, but also provided insights into the molecular mechanism of the TG cotton delayed the development of *A. gossypii*.

Supplementary material. Transcriptome data validation (Figure. S1); Cumulative mortality and cumulative reproduction of *A. gossypii* after spraying dsJHAMT. (Figure. S2). Date control statistics of transcriptome of *A. gossypii* (Table S1); Primer sequences (Table S2). The supplementary material for this article can be found at https://doi.org/10.1017/S000748532400049X.

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References

Cai R, Tao G, Zhao P, Xia QY, He HW and Wang YJ (2022) POU-M2 promotes juvenile hormone biosynthesis by directly activating the transcription of juvenile hormone synthetic enzyme genes in *Bombyx mori*. *Open Biology* **12**, 220031.

Champlin DT and Truman JW (1998) Ecdysteroids govern two phases of eye development during metamorphosis of the moth *Manduca sexta*. *Development (Cambridge, England)* **125**, 2009–2018.

Chen Y (2013) *Effects of juvenile hormone on reproduction and longevity in Helicoverpa armigera, and on the development of its F1 generation*. Huazhong Agricultural University

Cheng YB, Li YM, Li WR, Song YY, Zeng RS and Lu K (2020) Effect of hepatocyte nuclear factor 4 on the fecundity of *Nilaparvata lugens*: insights from RNA interference combined with transcriptomic analysis. *Genomics* **112**, 4585–4594.

Cheng SH, Li R, Chen ZB, Ni JP, Lv N, Liang PZ, Guo TF, Zhen CA, Liang P and Gao XW (2023) Comparative susceptibility of *Aphis gossypii* glover (Hemiptera: Aphididae) on cotton crops to imidacloprid and a novel insecticide cyproflanilide in China. *Industrial Crops and Products* **192**, 116053.

Daimon T and Shinoda T (2013) Function, diversity, and application of insect juvenile hormone epoxidases (CYP15). *Biotechnology and Applied Biochemistry* **60**, 82–91.

Daimon T, Kozaki T, Niwa R, Kobayashi I, Furuta K, Namiki T, Uchino K, Banno Y, Katsuma S, Tamura T, Mita K, Sezutsu H, Nakayama M, Itoyama K, Shimada T and Shinoda T (2012) Precocious metamorphosis in the juvenile hormone-deficient mutant of the silkworm, *Bombyx mori*. *PLoS Genetics* **8**, e1002486.

Ebert TA and Cartwright BO (1997) Biology and ecology of *Aphis gossypii* Glover (Homoptera: Aphididae). *Southwest Entomologist* **22**, 116–153.

Eid AE, El-Heneidy AH, Hafez AA, Shalaby FF and Adly D (2018) On the control of the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), on cucumber in greenhouses. *Egyptian Journal of Biological Pest Control* **28**, 64.

Fu KY, Li Q, Zhou LT, Meng QW, Lu FG, Guo WC and Li GQ (2016) Knockdown of *juvenile hormone acid methyl transferase* severely affects the performance of *Leptinotarsa decemlineata* (Say) larvae and adults. *Pest Management Science* **72**, 1231–1241.

Guncan A, Madanlar N, Yoldas Z, Ersin F and Tuzel Y (2006) Pest status of organic cucumber production under greenhouse conditions in Izmir (Turkey). *Turkish Journal of Entomology* **30**, 183–193.

Hiruma K and Kaneko Y (2013) Hormonal regulation of insect metamorphosis with special reference to juvenile hormone biosynthesis. *Current Topics in Developmental Biology* **103**, 73–100.

Jaiwal A, Natarajaswamy K and Rajam MV (2020) RNA silencing of hormonal biosynthetic genes impairs larval growth and development in cotton bollworm, *Helicoverpa armigera*. *Journal of Biosciences* **45**, 109.

Jindra M, Palli SR and Riddiford LM (2013) The juvenile hormone signaling pathway in insect development. *Annual Review of Entomology* **58**, 181–204.

Kinjoh T, Kaneko Y, Itoyama K, Mita K, Hiruma K and Shinoda T (2007) Control of juvenile hormone biosynthesis in *Bombyx mori*: cloning of the enzymes in the mevalonate pathway and assessment of their developmental expression in the corpora allata. *Insect Biochemistry and Molecular Biology* **37**, 808–818.

Kola VS, Renuka P, Madhav MS and Mangrauthia SK (2015) Key enzymes and proteins of crop insects as candidate for RNAi based gene silencing. *Frontiers in Physiology* **6**, 119.

Lenaerts C, Van Wielendaele P, Peeters P, Vanden Broeck J and Marchal E (2016) Ecdysteroid signalling components in metamorphosis and development of the desert locust, *Schistocerca gregaria*. *Insect Biochemistry and Molecular Biology* **75**, 10–23.

Li K, Jia QQ and Li S (2019) Juvenile hormone signaling - a mini review. *Insect Science* **26**, 600–606.

Livak KJ and Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\triangle\triangle Ct}$ method. *Methods (San Diego, Calif.)* **25**, 402–408.

Marchal E, Zhang JR, Badisco L, Verlinden H, Hult EF, Van Wielendaele P, Yagi KJ, Tobe SS and Vanden Broeck J (2011) Final steps in juvenile hormone biosynthesis in the desert locust, *Schistocerca gregaria*. *Insect Biochemistry and Molecular Biology* **41**, 219–227.

Minakuchi C, Namiki T, Yoshiyama M and Shinoda T (2008) RNAi-mediated knockdown of *juvenile hormone acid O-methyltransferase* gene causes precocious metamorphosis in the red flour beetle *Tribolium castaneum*. *Febs Journal* **275**, 2919–2931.

Mirth CK, Tang HY, Makohon-Moore SC, Salhadar S, Gokhale RH, Warner RD, Koyama T, Riddiford LM and Shingleton AW (2014) Juvenile hormone regulates body size and perturbs insulin signaling in Drosophila. *Proceedings of The National Academy of Sciences of The United States of America* **111**, 7018–7023.

Navale PM, Manamohan M, Asokan R, Krishna V, Sharath CG, Prasad BK, Latha J, Krishna KNK and Ellango R (2017) Transgenic tomato expressing dsRNA of juvenile hormone acid O-methyl transferase gene of *Helicoverpa armigera* (Lepidoptera: Noctuidae) affects larval growth and its development. *Journal of Asia-Pacific Entomology* **20**, 559–567.

Niwa R, Niimi T, Honda N, Yoshiyama M, Itoyama K, Kataoka H and Shinoda T (2008) Juvenile hormone acid O-methyltransferase in *Drosophila melanogaster*. *Insect Biochemistry and Molecular Biology* **38**, 714–720.

Oi CA, Ferreira HM, Silva RCD, Bienstman A, Nascimento FSD and Wenseleers T (2021) Effects of juvenile hormone in fertility and fertility-signaling iworkers of the common wasp *Vespula vulgaris*. *PLoS One* **16**, e0250720.

Orth AP, Lan Q and Goodman WG (1999) Ligand regulation of juvenile hormone binding protein mRNA in mutant *Manduca sexta*. *Molecular and Cellular Endocrinology* **149**, 61–69.

Pan YO, Wen SY, Chen XW, Gao XW, Zeng XC, Liu XM, Tian FY and Shang QL (2020) UDP-glycosyltransferases contribute to spirotetramat resistance in *Aphis gossypii* Glover. *Pesticide Biochemistry and Physiology* **166**, 104565.

Riddiford LM (2020) *Rhodnius*, golden oil, and *Met*: a history of juvenile hormone research. *Frontiers in Cell and Developmental Biology* **8**, 679.

Riddiford LM, Hiruma K, Zhou XF and Nelson CA (2003) Insights into the molecular basis of the hormonal control of molting and metamorphosis from *Manduca sexta* and *Drosophila melanogaster*. *Insect Biochemistry and Molecular Biology* **33**, 1327–1338.

Shen Y, Chen YZ and Zhang CX (2021) RNAi-mediated silencing of ferritin genes in the brown planthopper *Nilaparvata lugens* affects survival, growth and female fecundity. *Pest Management Science* **77**, 365–377.

Staal GB (1975) Insect growth regulators with juvenile hormone activity. *Annual Review of Entomology* **20**, 417–460.

Staal GB (1986) Anti juvenile hormone agents. *Annual Review of Entomology* **31**, 391–429.

Tian Z, Guo S, Li JX, Zhu F, Liu W and Wang XP (2021) Juvenile hormone biosynthetic genes are critical for regulating reproductive diapause in the cabbage beetle. *Insect Biochemistry and Molecular Biology* **139**, 103654.

Tibbetts EA, Izzo A and Tinghitella RM (2011) Juvenile hormone titer and advertised quality are associated with timing of early spring activity in *Polistes dominulus* foundresses. *Insectes Sociaux* **58**, 473–478.

Ullah F, Gul H, Wang X, Ding Q, Said F, Gao XW, Desneux N and Song DL (2020) RNAi-mediated knockdown of chitin synthase 1 (*CHS1*) gene causes mortality and decreased longevity and fecundity in *Aphis gossypii*. *Insects* **11**, 22.

Wei LY, Zhang LJ, Liu XN, Gao XW and Liu N (2021) Effect of RNAi targeting *CYP6CY3* on the growth, development and insecticide susceptibility of *Aphis gossypii* by using nanocarrier-based transdermal dsRNA delivery system. *Pesticide Biochemistry and Physiology* **177**, 104878.

Wu KM and Guo YY (2005) The evolution of cotton pest management practices in China. *Annual Review of Entomology* **50**, 31–52.

Wumuerhan P, Guo PP, Ma SJ, Gao XW, Zhang LJ, Zhang S and Ma DY (2019) Resistance of different field populations of *Aphis gossypii* to ten insecticides in Xinjiang. *Plant Protection* **45**, 273–278.

Xu QY, Deng P, Mu LL, Fu KY, Guo WC and Li GQ (2019) Silencing Taiman impairs larval development in *Leptinotarsa decemlineata*. *Pesticide Biochemistry and Physiology* **160**, 30–39.

Xu ZY, Yan R, Qian JL, Chen DP, Guo YR, Zhu GN, Wu HM and Chen ML (2022) RNAi-mediated knockdown of juvenile hormone esterase causes mortality and malformation in *Tribolium castaneum*. *Entomological Research* **52**, 476–482.

Yamanaka N, Rewitz KF and O'Connor MB (2013) Ecdysone control of developmental transitions: lessons from Drosophila research. *Annual Review of Entomology* **58**, 497–516.

Ye C, Jiang YD, An X, Yang L, Shang F, Niu JZ and Wang JJ (2019) Effects of RNAi-based silencing of chitin synthase gene on moulting and fecundity in pea aphids (*Acyrthosiphon pisum*). *Scientific Reports* **9**, 3694.

Yin Y, Qiu YW, Huang J, Tobe SS, Chen SS and Kai ZP (2020) Enzymes in the juvenile hormone biosynthetic pathway can be potential targets for pest control. *Pest Management Science* **76**, 1071–1077.

Yu XD, Liu ZC, Huang SL, Chen ZQ, Sun YW, Duan PF, Ma YZ and Xia LQ (2016) RNAi-mediated plant protection against aphids. *Pest Management Science* **72**, 1090–1098.

Zeng X, Pan Y, Song J, Li J, Lv Y, Gao X, Tian F, Peng T, Xu H and Shang Q (2021) Resistance risk assessment of the ryanoid anthranilic diamide insecticide cyantraniliprole in *Aphis gossypii* Glover. *Journal of Agricultural and Food Chemistry* **69**, 5849–5857.

Zhang S, Wang Y, Zhou J, Li J, Luo YQ, Weng Q and Zong SX (2016) cDNA cloning and expression analysis of the juvenile hormone acid methyltransferase from seabuckthorn carpenterworm, *Holcocerus hippophaecolus* (Lepidoptera: Cossidae). *Entomological Research* **46**, 23–30.

Zhang HH, Chen A, Shan T, Dong WY, Shi XY and Gao XW (2020) Cross-resistance and fitness cost analysis of resistance to thiamethoxam in melon and cotton aphid (Hemiptera: Aphididae). *Journal of Economic Entomology* **113**, 1946–1954.

Zhang ZX, Ma YJ, Ma XY, Hu HY, Wang D, Song XP, Ren XL and Ma Y (2021) Combined transcriptomic analysis and RNA interference reveal the effects of methoxyfenozide on ecdysone signaling pathway of *Spodoptera exigua*. *International Journal of Molecular Sciences* **22**, 9080.

Zhang XS, Li S and Liu SN (2022a) Juvenile hormone studies in *Drosophila melanogaster*. *Frontiers in Physiology* **12**, 785320.

Zhang LJ, Wei YJ, Wei LY, Liu XN and Liu N (2022b) Effect of transgenic cotton lines expressing *dsAgCYP6CY3-P1* on the growth and detoxification ability of *Aphis gossypii* Glover. *Pest Management Science* **79**, 481–488.

Zhao J, Zhou YL, Li X, Cai WL and Hua HX (2017) Silencing of juvenile hormone epoxide hydrolase gene (Nljheh) enhances short wing formation in a macropterous strain of the brown planthopper, *Nilaparvata lugens*. *Journal of Insect Physiology* **102**, 18–26.

Zheng JY, Wu PZ, Huang Y, Zhang Y and Qiu LH (2024) Identification of insect cuticular protein genes *LCP17* and *SgAbd5* from *Helicoverpa armigera* and evaluation their roles in fenvalerate resistance. *Pesticide Biochemistry and Physiology* **199**, 105775.

Zhou QH, Zhang Q, Yang RL, Yuan GR, Wang JJ and Dou W (2022) RNAi-mediated knockdown of juvenile hormone acid O-methyltransferase disrupts larval development in the oriental fruit fly, *Bactrocera dorsalis* (Hendel). *Pesticide Biochemistry and Physiology* **188**, 105285.