EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
*Eur. J. Entomol.* **122**: 65–72, 2025
doi: 10.14411/eje.2025.007
ORIGINAL ARTICLE

# Cloning and expression analysis of a detoxification enzyme *BmmGSTo3* gene in wild silkworm, *Bombyx mandarina* (Lepidoptera: Bombycidae)

**Ruonan Li[1,2]*, Enxi Chen[1,2]*, Yuming Zhang[1,2]*, Mengjiao Wang[1,2], Lin Zhu[1,2], Yixuan Qian[1,2], Guodong Zhao[1,2]** and Heying Qian[1,2]****

[1] Jiangsu Key Laboratory of Sericultural Biology and Biotechnology, School of Biotechnology, Jiangsu University of Science and Technology, No. 666 Changhui Road, Zhenjiang 212100, P.R. China; e-mails: 2361572462@qq.com, cnx1110k@163.com, chirszhang27@163.com, wangmengjiao001128@163.com, zl15680879232@163.com, qianyx0915@163.com, sdgdzhao@just.edu.cn, qianheying123@163.com

[2] Key Laboratory of Silkworm and Mulberry Genetic Improvement, Ministry of Agriculture and Rural Affairs, Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212100, China

**Key words.** BmNPV, insecticide, oxidative stress, virus, glutathione S-transferase

**Abstract.** *Bombyx mandarina* is one of prevalent pests in mulberry gardens during summer and autumn, posing significant challenges to sericulture across China. Glutathione S-transferase (GST), a vital detoxification enzyme in insects, plays a crucial role in protecting organisms from DNA damage and oxidative stress. In this study, the *GSTo3* gene, coding glutathione S-transferase of *B. mandarina*, was cloned and its physicochemical properties and structures were predicted using bioinformatics tools. The relative expression levels in various tissues and induced transcriptional levels were detected by using qRT-PCR. Results revealed a 99.17% sequence similarity of *GSTo3* gene between *B. mandarina* and *Bombyx mori*. It is mainly distributed in the head, fat body and epidermis tissues of *B. mandarina* larvae. After BmNPV infection, elevated *GSTo3* expression levels were observed in the midgut. The transcriptional levels of *GSTo3* gene were significantly up-regulated after exposure to phoxim, deltamethrin and chlorfenapyr exposure respectively in the fat body and midgut of *B. mandarina*. These findings underscore the significance of *GSTo3* gene in exogenous materials metabolism and provide a new perspective on the resistance mechanism in *B. mandarina*.

## INTRODUCTION

*Bombyx mandarina*, an important pest in mulberry gardens, feeds on mulberry leaves and was widespread across China, becoming one of the main insect pests in mulberry gardens during summer and autumn (Yokoyama et al., 2021). The larvae of *B. mandarina* feed on young mulberry leaves, often resulting in extensive damage to mulberry trees (Kumar et al., 2019). *B. mandarina* has strong resistance and tolerance in the wild environment, making it is difficult to control (Wan et al., 2021). Moreover, *B. mandarina* not only causes significant damage to mulberry trees but also impacts tree formation and indirectly transmits microparticle diseases through feces and residual liquid to *Bombyx mori*, especially through cross-infection, which poses hidden dangers to *Bombyx mori* breeding (Zhang, 2016). The pupae of *B. mandarina* exhibit diapause, and the hatching of eggs is prolonged, resulting in asynchronous group development and the occurrence of overlapping generations (Huang, 1985). The wild silkworm *B. mandarina* living in the natural environment, has strong stress resistance and adaptability after harsh natural selection (Kumar et al., 2019). Although most *B. mandarina* are susceptible to insecticides, some with strong resistance or in a specific growth stage (such as the egg or pupal stage) can escape the harm of insecticides, which is another main reason why *B. mandarina* is difficult to control (Shen et al., 2003). Chinese *B. mandarina* are closely related to Chinese *Bombyx mori*. Japanese *B. mandarina* have 27 chromosomes per haploid genome, while Chinese *B. mandarina* carry 28 chromosomes, the same number as domestic *Bombyx mori* (Banno et al., 2004).

*Bombyx mandarina* can also be infected by *Bombyx mori* nuclear polyhedrosis virus (BmNPV), a common virus in the sericulture industry, leading to hematogenous pyosis of silkworms (Ding et al., 2023a). *B. mandarina* can contaminate mulberry leaves through feces, residual liquid, etc., and can indirectly transmit diseases to domestic silkworms, especially the cross-infection of BmNPV to domestic silk-

* R.L., E.C. and Y.Z. contributed equally to this work.

** Corresponding authors; e-mails: sdgdzhao@just.edu.cn, qianheying123@163.com

worms, which will bring great harm to sericulture (Ding et al., 2023b). O, O-diethyl O-(alpha-cyanobenzylideneamino) phosphorothioate (phoxim) is an effective organic phosphorus pesticide widely used in agriculture due to its broad-spectrum activity (Kurauchi et al., 2009). Phoxim exposure mainly inhibits the activity of acetylcholinesterase, leading to the accumulation of acetylcholine in the postsynaptic membrane and the eventual death of pests (Li et al., 2016). Deltamethrin insecticides are characterized by high activity, a wide insecticidal spectrum, fast efficacy, and low toxicity to humans and animals (Saillenfait et al., 2015). Deltamethrin is a type II synthetic pyrethroid used worldwide as an insecticide and acaricide (Anadón et al., 2009). Deltamethrin is renowned for its high toxicity to insects. It exerts contact and stomach toxicity, delivers rapid knockdown effects, and lacks fumigant and systemic action. Moreover, it exhibits repellent properties against certain pests at elevated concentrations (Yousef et al., 2006). Chlorfenapyr is a pyrrole insecticide whose insecticidal mechanism mainly interferes with the mitochondrial oxidative phosphorylation process in insect cells, leading to the disruption of ATP production and cell death (Black et al., 1994). However, many insects are becoming more resistant to such pesticides.

Insects acquire resistance by two main mechanisms: reducing the sensitivity of acetylcholinesterase (AChE) to organophosphorus insecticides and increasing the activity of enzymes hydrolyzing and isolating organophosphorus pesticides, including estinases (EST), cytochrome P450 monooxygenase, and glutathione S-transferase (Hafeez et al., 2022). Studies have shown that excessive expression of detoxification enzymes and enhanced product activity are the main causes of resistance to pyrethroids and organophosphorus insecticides in insects (Papadopoulos et al., 2000). Changes in the expression level or gene structure of detoxification enzymes directly lead to corresponding changes in their detoxification capacity. Numerous studies have demonstrated a close association between the overexpression of induced detoxification genes and insecticide resistance in insects (Zhang et al., 2018). Glutathione S-transferase is a multifunctional phase II enzyme that plays a central role in the detoxification of endogenous and heterologous biological compounds (Zuo et al., 2007; Koirala et al., 2022). High resistance to insecticides in insect pests is associated with high GST enzyme activity (Patra et al., 2022). It has been shown that the *GSTd1*, *GSTo3*, and *GSTs1* genes in *Bombyx mori* are involved in the response to many exogenous substances (Wang et al., 2013). Studies have shown that *GSTo1* may play a role in organophosphorus pesticide resistance (Pavlidi et al., 2018). Insects typically exhibit the highest expression of GSTs in vital detoxification tissues such as the fat body and midgut (Yu et al., 2008). Additionally, GSTs are distributed in other tissues such as the epidermis, Malpighian tubules, and head (Zhao et al., 2011). Understanding the important role of the detoxification enzyme family in the metabolism and resistance to insecticides such as phoxim, deltamethrin, and chlorfenapyr makes it crucial.

In this study, a glutathione S-transferase *GSTo3* gene of *B. mandarina* was cloned and sequenced, and the secondary and tertiary structures of its related amino acids were predicted. Then the changes in *GSTo3* gene expression in 3rd day 5th instar stage *B. mandarina* were determined after feeding three insecticides, namely phoxim, deltamethrin and chlorfenapyr, for 24 and 48 h. The expression levels of *GSTo3* gene in eight tissues of normal silkworm as well as two tissues inducted by BmNPV were detected. The functional characteristics of glutathione S-transferases in *B. mandarina* were predicted by analyzing the changes of *GSTo3* gene expression in different situations. The results showed that the *B. mandarina* detoxification enzyme GSTo3 has a high degree of homology and specificity in the detoxification process of insects. The results of quantitative PCR showed high expression of *GSTo3* in the fat body and midgut tissues of *B. mandarina*, suggesting that it plays an important role in pesticide metabolism and detoxification processes. The expression of glutathione-S-transferase *GSTo3* changed in different pesticide treatment groups, confirming its important role in pesticide metabolism (Yu et al., 2008). In addition, lepidopteran insects play important ecological roles in ecosystems, such as the hierarchical relationships in pollination and the food chain (Wang et al., 2016). The study on effects of pesticides on these insects will enhance our understanding of their broader impact on ecosystems, offering a scientific foundation for environmental protection and sustainable agriculture.

## MATERIAL AND METHODS

### Insects

The larvae of *B. mandarina* used in this research were provided by the Sericulture Research Institute of the Chinese Academy of Agricultural Sciences and raised mulberry leaves at 25°C ± 1°C and 60%–75% relative humidity on a 12L : 12D cycle under standard conditions.

### Chemicals and reagents

The phoxim pesticides for the experiment were obtained from Guangzhou Yinanong Biochemical Company Limited, Guangzhou City, Guangdong Province, China, and the deltamethrin pesticides were obtained from Bayer CropScience Co (China). The chlorfenapyr pesticides were obtained from Hercules Biotechnology Company, Jiangxi Province, China.

### Pesticide and BmNPV treatment

Reagent preparation: To create a 500 ml solution with a concentration of 4 μg/mL, combine 2 ml of the original phoxim solution with 498 ml of distilled water. Then, to achieve a concentration of 0.4 μg/mL, dilute 2 ml of the previously prepared solution with 498 ml of distilled water (Peng et al., 2011). Deltamethrin and chlorfenapyr were diluted according to this method, and the concentrations of deltamethrin were 0.02 mg/L and 0.002 mg/L; the diluted concentration of chlorfenapyr was 100 mg/L and 10 mg/L (Chai et al., 2010). The diluted solution was used as the working solution. Mulberry leaves were immersed in 500 ml of the working solution for 1 min and air dried. Mulberry leaves treated with distilled water were used as a control, and 3rd day 5th instar stage larvae were fed with three kinds of insecticides for 24 h and 48 h.

The 3rd day 5th instar stage larvae of *B. mandarina* were fed with mulberry leaves impregnated with a concentration of $1.0 \times 10^8$

POBs/mL BmNPV (POBs: polyhedral occlusion bodies) suspension for 8 h and then switched to fresh non-toxic mulberry leaves, with mulberry leaves impregnated with distilled water fed at the same time as a control group. Five larvae were taken from each group at each time point after 24 h, 48 h and 72 h of treatment (Yu et al., 2022).

### Extraction of total RNA

After 24 h of mulberry leaf treatment, a part of *B. mandarina* was separated from fat body and midgut tissue and stored at –80°C. Total RNA was extracted from tissues using the Nanjing Pro FreeZol Reagent kit and reverse transcribed using the Nanjing Pro HiScript II 1st Strand cDNA Synthesis Kit. The above procedure was repeated after 48 h. The head, hemolymph, Malpighian tubule, midgut, fat body, posterior silk glands, middle silk glands and epidermis of *B. mandarina* were taken, and the total RNA was extracted from the tissue using the FreeZol Reagent kit and reverse transcribed using the HiScript II 1st Strand cDNA Synthesis Kit. BmNPV was used to infect *B. mandarina*, and the above operation was repeated after 24, 48 and 72 h; for qPCR, PerfectStart® Green qPCR SuperMix reagent of Beijing Total Gold Biotechnology Co., Ltd. was used.

### PCR amplification of the coding region of *BmmGSTo3*

With reference to the sequence information of the coding region of the glutathione S-transferase gene obtained from the NCBI database (DQ443293), the primers required for the experiment were produced by Novizan Biotechnology Co., Ltd. (Nanjing). The midgut cDNA from *B. mandarina* was used as a template for PCR amplification. PCR amplification system: 94°C pre-denaturation for 5 min; 95°C denaturation for 20 s, 48°C annealing for 20 s (annealing temperature according to primer design), 72°C extension for 5 min, 32 cycles; 72°C final extension for 10 min, 4°C preservation. Quantitative PCR with a total of 20 µL of system reaction, and the program was as follows: pre-denaturation at 95°C for 3 min; denaturation at 95°C for 10 s, annealing at 60°C for 30 s, 40 cycles; 95°C for 15 s, 60°C for 60 s, 95°C for 15 s. PCR reactions were performed with three technical replicates. SnapGene software was used to design the amplification primers (Table 1).

### Construction of the phylogenetic tree

Using MEGA-X to construct phylogenetic trees based on GSTo3 amino acid sequences of *B. mandarina* and other insects by the neighbor-joining method for 1,000 times. The online website iTOL (https://itol.embl.de) is used to decorate the phylogenetic tree.

### Statistical analysis

Statistical analysis was performed using SPSS software, paired t-test (two-tailed) and significant difference analysis. Differences in tissue expression after insecticide treatment were considered statistically significant. The * indicates significant difference ($P < 0.05$), ** indicates very significant difference ($P < 0.01$) and *** indicates the difference is super significant ($P < 0.001$).

## RESULTS

### Cloning and sequence characterisation of *GSTo3* gene in *B. mandarina*

The sequence of coding region of the glutathione S-transferase (*GSTo3*) gene in *B. mandarina* was obtained by PCR amplification, and sequencing service was provided by Sangon Biotech (Shanghai) Co., Ltd. The open reading frame (ORF) of the gene was verified by sequencing to be 723 bp long, encoding 240 amino acids (Fig. 1). The predicted molecular formula of the gene was $C_{1311}H_{1984}N_{346}O_{358}S_{9.}$ There were no transmembrane regions and signal peptides. In comparison with the CDS sequence of *GSTo3* gene, the CDS sequence of *GSTo3* gene was 99.17% similar to the CDS sequence of *Bombyx mori GSTo3* gene (Fig. 1). In comparison with the amino acid sequence of *GSTo3* gene of other species, the amino acid sequence encoded by *GSTo3* in *B. mandarina* has high homology with insects of *Bombyx mori*, *Pailio machano*, *Paolio xuthus*, *Cnaphalo crocis* and *Ostrinia furnacalis*, but lower homology with mammals and plants. These results indicate that the protein encoded by the *GSTo3* gene has structural features typical of insect glutathione S-transferase (Fig. 2).

### The amino acid phylogenetic tree of *GSTo3* gene of *B. mandarina*

Based on the GSTo3 amino acid sequence of *B. mandarina*, the phylogenetic tree was constructed by homology with the GSTo3 amino acid sequence of *Bombyx mori*, *Pailio machano*, *Paolio xuthus*, *Cnaphalo crocis*, *Ostrinia furnacalis*, *Cydia pomonella*, *Spodoptera exigua* and *Helicoverpa armigera*. The results showed that the amino acid sequence of *B. mandarina* GSTo3 was closely related to the amino acid sequence of *Bombyx mori*, *Pailio machano*, *Paolio xuthus*, *Cnaphalo crocis* and *Ostrinia furnacalis*, but far related to the amino acid sequence of *Cydia pomonella*, *Spodoptera exigua* and *Helicoverpa armigera* (Fig. 3).

### Protein structure prediction of *GSTo3* gene of *B. mandarina*

The secondary structure and the tertiary structure of the *B. mandarina* GSTo3 protein were predicted in https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_gor4.html and https://swissmodel.expasy.org respectively. The sequence analysis of amino acid secondary structure showed that the gene encoded 240 amino acids, containing 37.08% of random coil, 41.67% of α-helix, 15.83% of extended strand and 5.42% of β-turn (Fig. 4).

The results demonstrated significant homology of the GSTo3 protein, with characteristic features typical of the

**Table 1.** Primers associated with PCR.

| Primer name | Primer sequence (5'–3') | Tm (°C) | Product length (bp) |
|---|---|---|---|
| Actin3-F | CGGCTACTCGTTCACTACC | 59.72 | 147 |
| Actin3-R | CCGTCGGGAAGTTCGTAAG | 59.72 | |
| BmmGSTo3-F | ATGACTTATTTTCACTCCGTAAACG | 50.8 | 723 |
| BmmGSTo3-R | CTACAAATAATAGCCCATAGAATGC | 52.5 | |
| BmmGSTo3-qPCR-F | TCTGCGACTACCTGGATG | 53.9 | 145 |
| BmmGSTo3--qPCR-R | TGGATGTATCTGCTCACTTC | 51.2 | |


**Fig. 1.** CDS sequence comparison of *GSTo*3 gene between *B. mandarina* and domestic *Bombyx mori.* Note: The black background base similarity is 100%; the dark gray background base similarity is 100% to 75%; and the gray background base similarity is 75% to 50%.

glutathione S-transferase family, including the N-terminal sequence, the central glutathione-binding domain, and a phosphorylation site at the C-terminal. Furthermore, the sequence exhibited several conserved amino acid motifs, indicating evolutionary conservation.

## Expression levels of *BmmGSTo3* gene in different tissues

The expressions of *GSTo3* at transcriptional level were detected by using real-time quantitative PCR. The results revealed that the expression levels of *GSTo3* varied significantly in different tissues of the 3rd day 5th instar stage larvae. It was highly expressed in the head, middle silk gland, and epidermis of 5th instar larvae, with the highest expression in the epidermis and relatively low expression levels in other tissues (Fig. 5).

## Effect of feeding BmNPV on the expression level of *BmmGSTo3* gene

To explore the expression level changes of *BmmGSTo3* gene in response to BmNPV in *B. mandarina*, real-time fluorescence quantitative PCR was used for determination. The relative expression level changes of *BmmGSTo3* gene in hemolymph and midgut were detected at 24 h, 48 h, and 72 h after feeding BmNPV. In hemolymph, the expression levels of *BmmGSTo3* gene were all up-regulated at 24, 48, and 72 h after treatment with no significance, while the expression in the midgut was increased by 1.60, 1.80, and 5.63 times at the same time points. Interestingly, due to the massive amplification of the virus at 72 h after feeding, the transcriptional level of *BmmGSTo3* gene was increased significantly (Fig. 6). These results indicated that

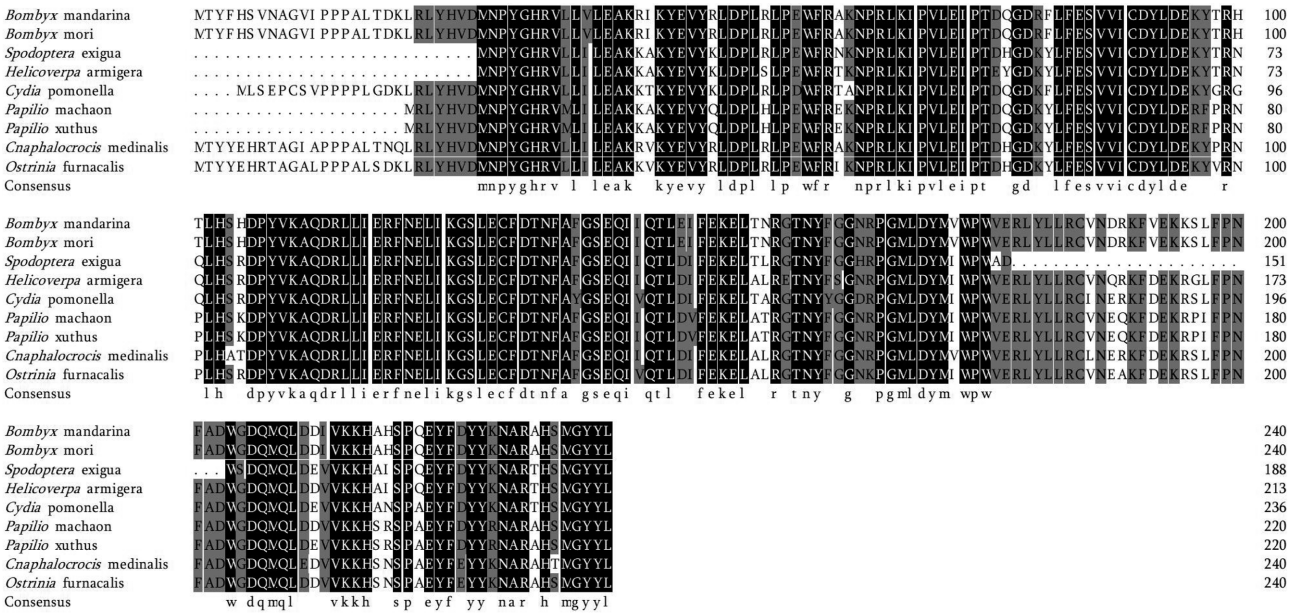


**Fig. 2.** Comparison of the amino acid sequence of the *B. mandarina GSTo*3 protein with the sequences of other species. Note: The black background base similarity is 100%; the dark gray background base similarity is 100% to 75%; and the gray background base similarity is 75% to 50%.

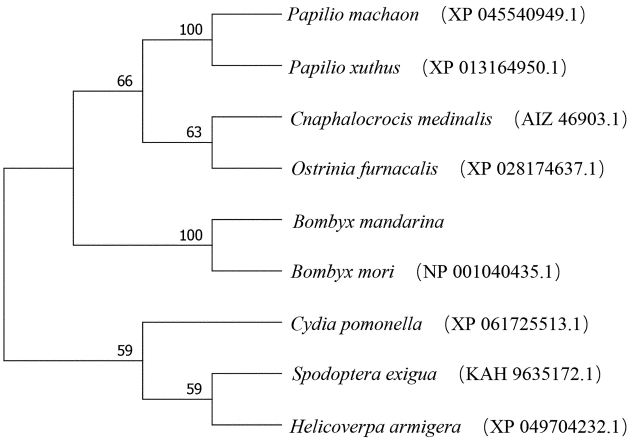


**Fig. 3.** Phylogenetic tree of *GST*o3 proteins of *B. mandarina* and other species based on amino acid sequences constructed by neighbor-joining method (1000 replicates).

BmNPV virus infection had a significant effect on *GSTo3* gene expression in *B. mandarina*, and the expression level increased with increasing infection time. However, the specific antiviral mechanism of GSTo3 in *B. mandarina* remains unclear, providing a direction for future studies.

### Expression of *BmmGSTo3* gene can be induced by insecticides

As a kind of detoxification enzyme, glutathione S-transferase may be involved in metabolism of exogenous substances such as insecticides. In this study, three kinds of common insecticides (phoxim, deltamethrin and chlorfenapyr) were chosen to examine the effects on the expression of *BmmGSTo3* gene. As shown in Fig. 7, the expression of *BmmGSTo3* gene can be induced by all three kinds of insecticides in both the fat body and midgut. However, an interesting result was that the expression of *BmmGSTo3* was up-regulated at 24 h after adding a low concentration of phoxim, while it was down-regulated by high concentration of phoxim (Fig. 7D). The same trends appeared after deltamethrin treatment (Fig. 7E). Two concentrations of chlorfenapyr can significantly induce the expression of *BmmGSTo3*, and a certain concentration effect was presented (Fig. 7C and Fig. 7F).

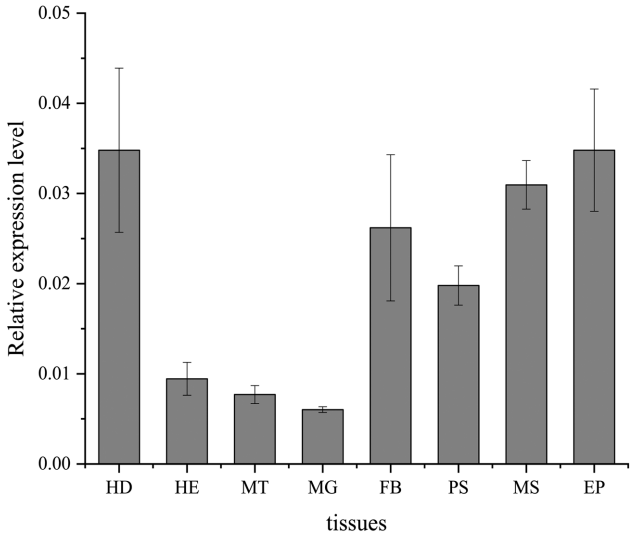


**Fig. 5.** Relative expression level of *BmmGST*o3 gene in different tissues of 5th instar larvae of *B. mandarina*. The results are expressed as mean S.E. HD – head; HE – hemolymph; MT – malpighian tubule; MG – midgut; FB – fat body; PS – posterior silk gland; MS – middle silk gland; EP – epidermis. Values represent means ± SEM (*N* = 3).

## DISCUSSION

Using genetic data obtained from the NCBI database for the indigenous insect *B. mandarina*, we successfully cloned the *GSTo3* gene in *B. mandarina*. This gene exhibits typical characteristics of the GST family, including conserved N-terminal and C-terminal sequences (Vaish et al., 2020). Sequence comparison at the amino acid level revealed a striking similarity between *B. mandarina GSTo3* gene and its homolog in *Bombyx mori*, with a 99.17% identity and this is in agreement with the results of previous studies (Gonis et al., 2022). Additionally, analysis of the predicted amino acid secondary structure of GSTo3 in *B. mandarina* indicated the absence of a signal peptide, which suggests that it may be a protein unrelated to signaling.

The main mechanisms of insecticide detoxification present in insects include reduced epidermal permeability, mutations at target sites, and increased levels of metabolic detoxification enzymes such as GST, P450 (Shao et al., 2021). In addition, previous studies have also demonstrat-

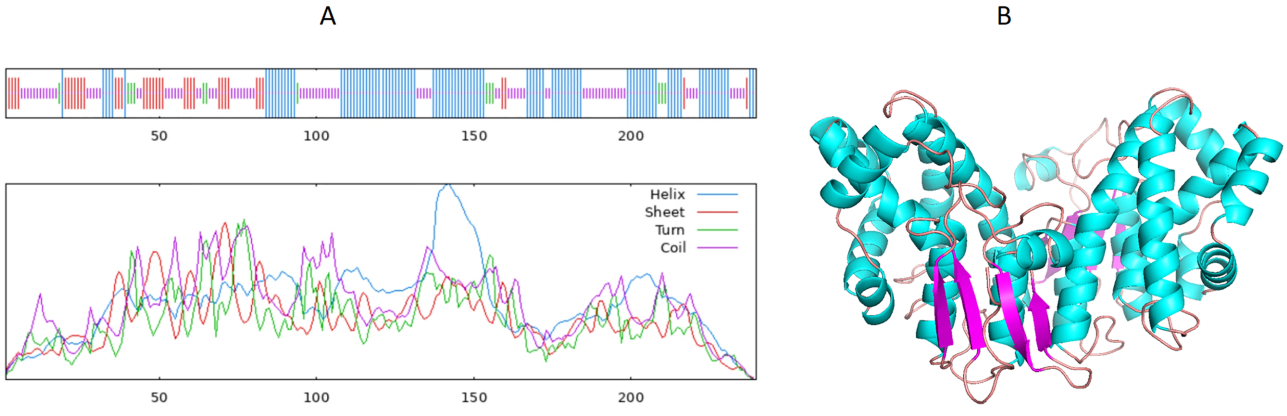


**Fig. 4.** Predicted two-dimensional structure of amino acids and protein three-dimensional structure based on *B. mandarina GST*o3 gene. A – secondary structure; B – three-dimensional structure.

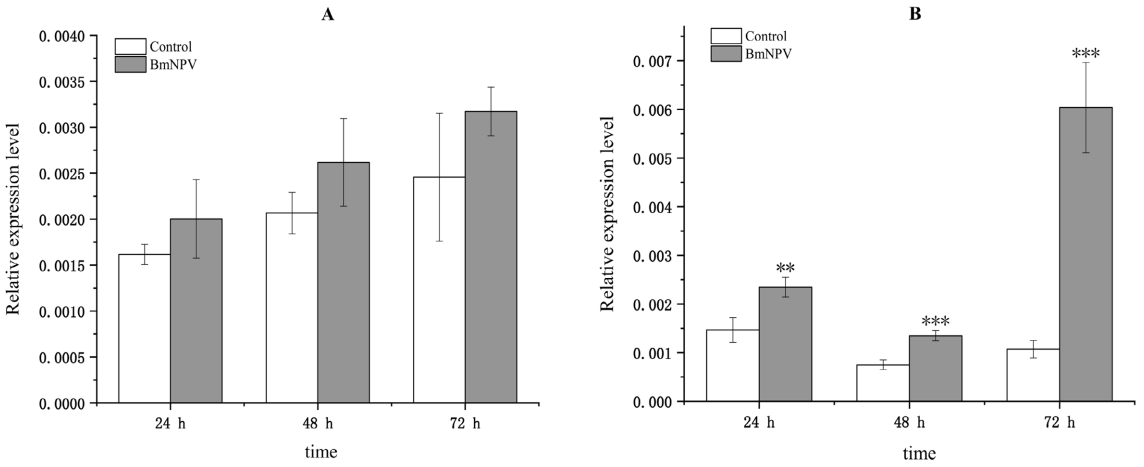


**Fig. 6.** Expression of *GSTo*3 gene in different tissues of *B. mandarina* in 3rd day 5th instar stage after feeding BmNPV. A – hemolymph; B – midgut. Asterisks denote significant differences between treatments and controls, as determined using pairwise T-test (**$p \leq 0.01$, ***$p \leq 0.001$). Values represent means ± SEM ($N = 3$).

ed that higher expression levels of *GST*s lead to insecticide tolerance or resistance in insects (Chen, 2017). After careful exploration in this study, it was found that the *GSTo3* gene of the *B. mandarina* is expressed in all eight tissues of the 3rd day 5th instar stage larvae. However, it is noteworthy that the transcription level of this gene is higher in the head and epidermis tissues than in other tissues, indicating that the *GSTo3* gene may play an important role in the detoxification of these tissues. Generally speaking, the *Bombyx mori* nuclear polyhedrosis virus infects *Bombyx mori* or *Bombyx mandarina* orally and enters various tissues and organs through the flow of hemolymph (Lin et al., 2024). The notable alterations in *GSTo3* expression observed in hemolymph and midgut of *B. mandarina* following BmNPV infection suggested a potential involvement of the *GSTo3* gene in response to viral infection. Specifically, numerous studies have demonstrated that the overexpression of induced detoxification genes is related to insect resistance to virus (Fang, 2010). Glutathione S-transferase significantly enhanced the antioxidant capacity of silkworms infected with BmNPV (Qian et al., 2022). This disclosed a novel avenue for our forthcoming investigations into the antiviral mechanisms of *B. mandarina*.

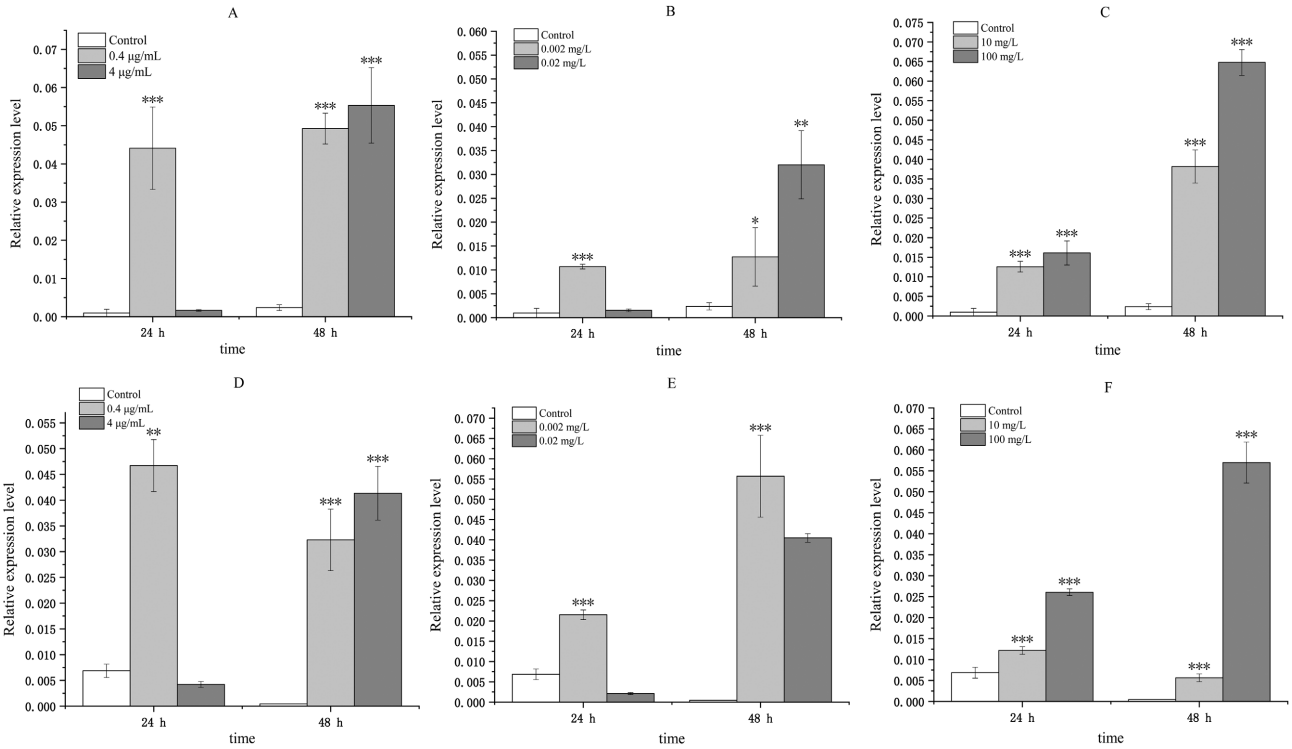


**Fig. 7.** Relative expression level of *BmmGST*o3 gene in different tissues of *B. mandarina* in 3rd day 5th instar stage after treatment with three kinds of pesticides. A – Phoxim treatment in fat body; B – Deltamethrin treatment in fat body; C – Chlorfenapyr treatment in fat body; D – Phoxim treatment in midgut; E – Deltamethrin treatment in midgut; F – Chlorfenapyr treatment in midgut. Asterisks denote significant differences between treatments and controls, as determined using pairwise T-test (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$). Values represent means ± SEM ($N = 3$).

The midgut and fat body are the main detoxification organs for insecticides in insects. The expression levels of detoxification enzymes in midgut and fat body were detected using real-time quantitative PCR. The results indicated that all three kinds of insecticides treatments led to a significant up-regulation of detoxification genes at 48 h after treatment, suggesting their involvement in the metabolism and detoxification of the three kinds of insecticides. Accordingly, we preliminarily speculated that this phenomenon may be closely related to the unique detoxification functions of these two tissues. The expression of *GSTo3* in the fat body and midgut of *B. mandarina* was elevated after the three kinds of insecticides, which is consistent with previous studies of *Bombyx mori* glutathione S-transferase (Yu et al., 2008). This further confirmed the important role of *GSTo3* in insecticide resistance in *B. mandarina*. The trend of *GSTo3* regulation was consistent in the phoxim and deltamethrin-treated groups, which may indicate that the two insecticides have similar effects on the expression levels of *GSTo3* gene in *B. mandarina* (Zhao et al., 2010). The increased expression of *GSTo3* gene was particularly significant in the chlorfenapyr-treated group, which may be related to the strong toxicity of chlorfenapyr on *B. mandarina*. Furthermore, our findings indicated a close correlation between insecticide resistance and the expression level of *GSTo3* gene. This presents a vital clue for further elucidating the detoxification and resistance mechanisms in *B. mandarina*. In addition, it is interesting that low concentrations of phoxim and deltamethrin cause upregulation of *GSTo3* at 24 h as opposed to high concentrations. We speculated that the upregulation of *GSTo3* expression induced by low concentrations of pesticides at 24 h may be a stress response, while high concentrations of pesticides may cause damage to the insect tissues. Interestingly, it recovered at 48 h after treatment.

In summary, the present study not only helped us to gain a deeper understanding of the detoxification and resistance mechanism of *B. mandarina*, but also provided an important theoretical basis for the practice of pest control and the development of new insecticides. Meanwhile, this study also provides a useful reference for further exploring the adaptation mechanism of *B. mandarina* in response to environmental pressure. Future research endeavors could focus on the functional validation of the *GSTo3* gene in *B. mandarina* and elucidate its regulatory mechanisms under diverse environmental stressors. Such investigations will contribute to a more comprehensive understanding of *B. mandarina* biology and its interactions with the environment, thus furnishing more scientifically sound strategies for pest management and ecological preservation.

**DECLARATION OF COMPETING INTEREST.** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**ACKNOWLEDGEMENTS.** This research was funded by Supported by China Agriculture Research System of MOF and MARA, grant number CARS-18-ZJ0101, Key R & D plan of Jiangsu Province (Modern Agriculture)-BE2020418 and Postgraduate Research & Practice Innovation Program of Jiangsu Province, grant number SJCX24_2582.

## REFERENCES

Anadón A., Martínez-Larrañaga M.R. & Martínez M.A. 2009: Use and abuse of pyrethrins and synthetic pyrethroids in veterinary medicine. — *Vet. J.* **182**: 7–20.

Banno Y., Nakamura T., Nagashima E., Fujii H. & Doira H. 2004: M chromosome of the wild silkworm, *Bombyx mandarina* (n = 27), corresponds to two chromosomes in the domesticated silkworm, *Bombyx mori* (n = 28). — *Genome* **47**: 96–101.

Black B.C., Hollingworth R.M., Ahammadsahib K.I., Kukel C.D. & Donovan S. 1994: Insecticidal action and mitochondrial uncoupling activity of AC-303,630 and related halogenated pyrroles. — *Pestic. Biochem. Physiol*. **50**: 115–128.

Chai J.P., Xie D.Y. & Chen S. 2010: Toxicity test of a new insecticide fenfenapyr to *Bombyx mori*. — *Newsl. Sericult. Sci*. **41**: 16–18 [in Chinese].

Chen J.J., Zhang J., Wu S.Z., Zhang G.L., Zhang K.K. & Hu D.Y. 2016: Determination of lambda-cyhalothrin in apples and soil by gas chromatography. — *Chin. J. Chromatogr*. **34**: 1005–1010.

Ding J.Q., Chen E.X., Li R.N., Lin M.J., Qian H.Y. & Zhao G.D. 2023a: Characterization and functional analysis of small heat shock protein gene *HSP19.5* in *Bombyx mandarina*. — *ScienceAsia* **49**: 819–826.

Ding J.Q., Jiang Q.P., Chen E.X., Lin M.J., Qian H.Y. & Zhao G.D. 2023b: Cloning and expression of small heat shock protein gene *BmmHSP19.9* in *Bombyx mandarina* (Lepidoptera: Bombycidae). — *Sci. Sericult*. **49**: 481–489.

Fang S.M. 2010: Genomic studies of insect glutathione S-transferases and its mediated resistance. — *Newsl. Sericult. Sci.* **4**: 8 [in Chinese].

Gonis E., Fraichard S., Chertemps T., Hecker A., Schwartz M., Canon F. & Neiers F. 2022: Expression patterns of *Drosophila melanogaster* glutathione transferases. — *Insects* **13**: 612, 10 pp.

Hafeez M., Ullah F., Khan M.M., Li X., Zhang Z., Shah S. & Lu Y. 2022: Metabolic-based insecticide resistance mechanism and ecofriendly approaches for controlling of beet armyworm *Spodoptera exigua*: a review. — *Environ. Sci. Pollut. Res.* **29**: 1746–1762.

Huang E.T. 1985: Study on hatching and annual generation algebra of overwintering eggs of *Bombyx mandarina*. — *Acta Sericol. Sin.* **4**: 8–14.

Koirala B.K.S., Moural T. & Zhu F. 2022: Functional and structural diversity of insect glutathione S-transferases in xenobiotic adaptation. — *Int. J. Biol. Sci.* **18**: 5713–5723.

Kumar D., Sun Z.L., Cao G.L., Xue R.Y., Hu X.L. & Gong C.L. 2019: Study of gut bacterial diversity of *Bombyx mandarina* and *Bombyx mori* through 16S rRNA gene sequencing. — *J. Asia-Pac. Entomol.* **22**: 522–530.

Kurauchi M., Morise H. & Eki T. 2009: Using the nematode *Caenorhabditis elegans* daf-16 mutant to assess the lifespan toxicity of prolonged exposure to ecotoxic agents. — *J. Health Sci.* **55**: 796–804.

Li F.C., Hu J.S., Tian J.H., Xu K.Z., Ni M., Wang B.B., Shen W.D. & Li B. 2016: Effects of phoxim on nutrient metabolism and insulin signaling pathway in silkworm midgut. — *Chemosphere* **146**: 478–485.

Lin M.J., Qian Y.X., Chen E.X., Wang M.J., Ouyang G., Xu Y., Zhao G.D. & Qian H.Y. 2024: The *Bmtret*1 gene family and its

potential role in response to BmNPV stress in *Bombyx mori*. — *Int. J. Mol. Sci*. **25**: 402, 12 pp.

Papadopoulos A.I., Boukouvala E., Kakaliouras G., Kostaropoulos J. & Papadopoulou-Mourkidou E. 2000: Effect of organophosphate and pyrethroid insecticides on the expression of GSTs from *Tenebrio molitor* pupae. — *Pestic. Biochem. Physiol.* **68**: 26–33.

Patra B., Mandal S., Sahoo S.K. & Hath T.K. 2022: Higher activity of glutathione S-transferase enzyme is associated with field evolved resistance in *Empoasca flavescens* Fabricius. — *Int. J. Trop. Insect Sci.* **42**: 2887–2895.

Pavlidi N., Vontas J. & Thomas V.L. 2018: The role of glutathione S-transferases (GSTs) in insecticide resistance in crop pests and disease vectors. — *Curr. Opin. Insect Sci.* **27**: 97–102.

Peng G.D., Wang J.M., Ma L., Wang Y.H., Cao Y.Q., Shen W.D. & Li B. 2011: Transcriptional characteristics of acetylcholinesterase genes in domestic silkworms (*Bombyx mori*) exposed to phoxim. — *Pestic. Biochem. Physiol.* **101**: 154–158.

Qian H.Y., Guo H.M., Zhang X., Liu M.Z., Zhao G.D., Xu A.Y. & Li G. 2022: Metabolic characterization of hemolymph in *Bombyx mori* varieties after *Bombyx mori* nucleopolyhedrovirus infection by GC-MS-based metabolite profiling. — *Arch. Virol*. **167**: 1637–1648.

Saillenfait A.M., Ndiaye D. & Sabaté J.P. 2015: Pyrethroids: exposure and health effects – an update. — *Int. J. Hyg. Environ. Health* **218**: 281–292.

Shao Y., Xin X.D., Liu Z.X., Wang J., Zhang R. & Gui Z.Z. 2021: Transcriptional response of detoxifying enzyme genes in *Bombyx mori* under chlorfenapyr exposure. — *Pestic. Biochem. Physiol.* **177**: 104899, 8 pp.

Shen W.D., Li B., Wei Z.G., Chen Y.H. & Pang G. 2003: Comparative study of environmental adaptability between *Bombyx mandarina* and domestic *Bombyx mori*. — *Acta Sericol. Sin.* **29**: 5 [in Chinese].

Vaish S., Gupta D., Mehrotra R., Mehrotra S. & Basantani M.K. 2020: Glutathione S-transferase: a versatile protein family. — *3 Biotech.* **10**: 321, 19 pp.

Wan L.R., Zhou A.L., Xiao W.F., Zou B.X., Jiang Y.M., Xiao J.S., De C. & Zhang Y.H. 2021: Cytochrome P450 monooxygenase genes in the wild silkworm, *Bombyx mandarina*. — *PeerJ* **9**: e10818, 21 pp.

Wang Y.H., Gu Z.Y., Wang J.M., Sun S.S., Wang B.B., Jin Y.Q., Shen W.D. & Li B. 2013: Changes in the activity and the expression of detoxification enzymes in silkworms (*Bombyx mori*) after phoxim feeding. — *Pestic. Biochem. Physiol.* **105**: 13–17.

Wang R., Ding S., Lu X.L. & Song B. 2016: Multi-scale effects of agricultural landscape heterogeneity on pollinator diversity in the middle and lower reaches of the Yellow River: a case study of Gongyi city. — *Chin. J. Appl. Ecol.* **27**: 9.

Yokoyama T., Saito S., Shimoda M., Kobayashi M., Takasu Y., Sezutsu H., Kato Y., Tominaga M., Mizoguchi A. & Shiomi K. 2021: Comparisons in temperature and photoperiodic-dependent diapause induction between domestic and wild mulberry silkworms. — *Sci. Rep.* **11**: 8052, 9 pp.

Yousef M.I., Awad T.I. & Mohamed E.H. 2006: Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by vitamin E. — *Toxicology* **227**: 240–247.

Yu Q.Y., Lu C., Li B., Fang S.M, Zuo W.D., Dai F.Y., Zhang Z. & Xiang Z.H. 2008: Identification, genomic organization and expression pattern of glutathione S-transferase in the silkworm, *Bombyx mori*. — *Insect Biochem. Mol. Biol.* **38**: 1158–1164.

Yu L., Cao Y., Ge S., Xu A., Qian H. & Li G. 2022: Identification of key genes involved in resistance to early stage of BmNPV infection in silkworms. — *Viruses* **14**: 2405, 17 pp.

Zhang J. 2016: Morphological recognition and comprehensive control of *Bombyx mandarina*. — *Agric. Technol. Service* **33**: 1 [in Chinese].

Zhang Y.L., Liu B.S., Zhang Z.C., Wang L.H., Guo H.F., Li Z., He P., Liu Z.W. & Fang J.C. 2018: Differential expression of P450 genes and nAChR subunits associated with imidacloprid resistance in *Laodelphax striatellus* (Hemiptera: Delphacidae). — *J. Econ. Entomol.* **111**: 1382–1387.

Zhao G.D., Wei Z.G., Zhang Y.L., Gao R.N., Wang R.X., Zhang T., Bing L. & Shen W.D. 2010: Quantitative analysis of induced expression of *GST* genes in the wild mulberry silkworm, *Bombyx mandarina*. — *Acta Entomol. Sin.* **21**: 487–490.

Zhao G.D., Zhang Y.L., Gao R.N., Wang R.X., Zhang T., Li B., Zhang Y., Lu C.D., Shen W.D. & Wei Z.G. 2011: Quantitative analysis of expression of six *BmGST* genes in silkworm, *Bombyx mori*. — *Mol. Biol. Rep*. **38**: 4855–5861.

Zuo W.D., Yu Q.Y., Li B., Liu Z., Dai F.Y. & Lu C. 2007: Cloning, sequence analysis and expression pattern of glutathione transferase gene in the silkworm, *Bombyx mori*. — *J. Agric. Biotechnol.* **4**: 595–601.

Received November 8, 2024; revised and accepted March 9, 2025
Published online March 28, 2025