RNA Interference to Control Asian Corn Borer Using dsRNA from a Novel Glutathione-S-Transferase Gene of Ostrinia furnacalis (Lepidoptera: Crambidae)

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Abstract

Glutathione-S-transferases (GST) comprise a multifunctional protein superfamily, which plays important roles as detoxifiers and antioxidants in insects. The GST in Asian corn borer has not been previously characterized. In this study, we cloned, characterized, and expressed the complete GST genes from the midgut of Asian corn borer. Furthermore, we designed htL4440-OfGST vector to exploit this gene for RNA interference (RNAi) strategy to control this pest. A complete GST cDNA sequence in Asian corn borer was obtained by reverse transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends technology. The gene was 887bp in length and contained a 705bp open reading frame and 5′ UTR and 3′ UTR lengths of 89 and 93bp, respectively. The putative sequence encoded a putative 234 amino acid residue peptide and had a predicted molecular weight of ~26kDa. The GST protein of Asian corn borer is hydrophilic and may have a 30 amino acid signal peptide with a cleavage site between L30 and K31. A recombination vector pET28a-OfGST was constructed for purification and antibody preparation. Western blotting analysis showed that this protein reached the maximum expression level around 24 h in Asian corn borer larvae fed the plant toxin 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one. A second vector, htL4440-OfGST, was constructed to generate the dsRNA of the GST gene. A larval feeding bioassay showed that the expressed dsRNA significantly reduced the detoxification ability of Asian corn borer larvae and increased mortality rate up to 54%. Our data indicated that GST plays very important roles in detoxifying in Asian corn borer and can be used as an RNAi method to control this pest in the field.

Key words: Asian corn borer, glutathione-S-transferase gene (GST), RNA interference, dsRNA, pest control

Plants are resistant to insect feeding through various methods; at the same time, insects have evolved a range of defense mechanisms to overcome plant resistances. Many herbivores produce detoxification enzymes that can convert toxic phytocompounds into nontoxic forms. There are three main types of detoxification enzymes in insects: P450 monooxygenases, carboxylesterases, and glutathione-S-transferases (GSTs). GSTs make up a large, almost ubiquitous superfamily of enzymes and have many important physiological functions in metabolic processes, especially in the detoxication process of harmful compounds. These enzymes function mainly through the conjugation of glutathione to target molecules (Motoyama and WC 1980, Clark and Shamaan 1984, Sherratt and Hayes 2002).

GSTs play important roles in detoxifying and antioxidant effects in insects (Fournier et al. 1992). Previous studies show that these enzymes are the main cause of high levels of resistance to plant defense compounds and insecticides in many agriculturally-important pests (Enayati et al. 2005, Fonseca-González et al. 2011). Many phytocompounds may serve in the plant as chemical defenses against insects, being antifeedants, poisons or interferers of nutrient absorption (Rosenthal and Berenbaum 2012). Zhao et al. report that BmGSTd1 (a GST) gene transcription level in Bombyx mori (L.)
(Lepidoptera: Bombycidae) increases in tissues exposed to ingested sodium fluoride (Zhao et al. 2010b). In addition, the authors found that GST genes are involved in the detoxification of the pesticides, dichlorvos and deltamethrin, and that the GSTe2 and GSTe5 genes may be mainly responsible for detoxification of xenobiotics (Zhao et al. 2010a). Thus, the study of GSTs has important theoretical and practical significance to prevent and control agricultural pests.

Certain secondary metabolites of plants can induce increased GST activity in some insects, thereby enhancing their metabolic effects on toxic secondary substances. We have isolated individual GST genes to understand the molecular mechanism by which this enzyme detoxifies heterogeneous toxic substances. Many plants defend themselves against herbivorous insects such as Asian corn borer by producing toxic 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Phuong et al. 2018). In response to plant metabolites like gossypol, DIMBOA and rutin, insect GSTs activity may increase to modulate their toxic effects (Motoyama and WC. 1980). GST is a large family, and so far, many putative GST genes have been cloned from a variety of insects (Hemming 2000). There are six major subclasses in insects: Delta, Epsilon, Sigma, Theta, and Zeta. (Enayati et al. 2005, Friedman 2011). To our knowledge, this gene has not been reported in Asian corn borer (Ostrinia furnacalis). The identification of the gene can be complicated by the existence of plural isoforms, making it difficult to isolate high-purity monomers.

RNA interference (RNAi) can specifically inhibit gene expression, and has become a powerful tool of gene function research (Plasterk 1998, Waterhouse and Helliwell 2003). The phenomenon exists across kingdoms, including plants, fungi, and viruses (Napoli et al. 1990, Romano and Macino 1992, Obbard et al. 2009). It is also used to study gene function in insects such as Plutella xylostella, Spodoptera litura, and Manduca sexta (Matthew 2004, Levin et al. 2005). Currently, RNAi has wide applications in control of plant pests and diseases (Yin et al. 2009, Yin et al. 2010, Wang et al. 2011, Joga et al. 2016). We have previously developed microRNAs mimics for control of Spodoptera exigua (Zhang et al. 2015a). Using RNAi, Bautista et al. demonstrated the function of a P450 gene, CYP6B1, involved in the metabolic detoxification of an insecticide in P. xylostella larvae. By feeding larvae with in vitro synthesized dsRNA, the authors reported that the dsRNA significantly reduced the expression of the CYP6B1 gene and insecticid resistance (Bautista et al. 2009).

Our study sought to sequence the GST gene of Asian corn borer and demonstrate its application in an RNAi strategy for possible control methods of the pest. We used known conserved GST sequences in NCBI GenBank (Benson et al. 2013) to design degenerate primers to clone the target gene. After confirming the gene sequence, the primers OfGST-specific primers were designed to RACE the GST gene in Asian corn borer according to instructions of the RACE Kit (Takara Bio, Shanghai, China). The corrected GST gene was labeled as OfGST1. All the primers were designed using Primer Premier 5.0 (Premier Biosoft, http://www.premierbiosoft.com/).

Materials and Methods

Reagents

RNAiso reagent, Taq DNA polymerase, RACE Kit, and PCR/Gel extraction Kit were purchased from Takara Bio (Shanghai, China). Reagents for SDS-PAGE, fixing solution, developer solution, ECL Western Blotting Substrate, nitrocellulose membrane, and other analytical reagents were purchased from Applygen Technologies (Beijing, China). BSA and Tween 20 were purchased from Ameresco (Solon, OH); Freund’s complete adjuvant, Freund’s incomplete adjuvant, and IPTG were purchased from Sigma-Aldrich (St. Louis, MO). Secondary antibodies and β-actin antibody were purchased from Santa Cruz Biotechnology (Dallas, TX).

Asian Corn Borer and OfGST1 Gene Cloning

Asian corn borer were reared in the Key Laboratory of Molecular Biology of Heilongjiang Province (Heilongjiang, China) at 25°C under a 14:10 (L:D) h and 70% relative humidity as described by Zhang et al. (Zhang et al. 2011). Midguts were isolated from similar-appearing third instar larvae, the lumen wasrapid washed with 0.85% NaCl (w/v) to remove debris, and then stored in liquid nitrogen and kept at −80°C for later RNA extraction and purification (Zhang et al. 2017).

Total RNA of Asian corn borer midgut was extracted using RNAiso reagent, converted to cDNA using a cDNA Kit (Takara Bio) according to the manufacturer’s instructions and stored at −20°C. According to the known amino acid sequences of GST from Helicoverpa armigera (Accession no. ABK40535), Antitherea pernyi (Accession no. ACB36909), B. mori (Accession no. NP_001037546), and P. xylostella (Accession no. BAJ10978), a multiple sequence alignment (Clustal Omega) was performed to find conserved sequences to design a pair of degenerate primers, Of-GST-JB-P1 and Of-GST-JB-P2 (Table 1). PCR reactions were conducted in 50 μl PCR reaction mixes: 10 X Taq buffer 5 μl, 1.5 μl of forward and reverse primer (10 μM each), 4 μl of 2.5 mM dNTP, 1 μl of Ex-Taq polymerase (Takara Bio, Shanghai, China), 2 μl of the cDNA template, and added ddH2O to a final volume of 50 μl. The thermocycler program was as follows: 94°C for 3 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 10 min. The PCR products were ligated into the vector of pMD18-T cloning vector (Takara Bio, Shanghai, China) and transformed into E. coli DH5α for blue/white screening. The positive clones were verified by colony PCR using primers M13-47 and RV-M (Table 1) and sequenced in both directions (Life Technologies, Shanghai, China).

After confirming the GST gene, RACE specific primers were designed to clone the GST gene in Asian corn borer according to instructions of the RACE Kit (Takara Bio, Shanghai, China). The corrected GST gene was labeled as OfGST1. All the primers were designed using Primer Premier 5.0 (Premier Biosoft, http://www.premierbiosoft.com/).

Bioinformatics Analysis

The phylogenetic tree of OfGST1 and its closely related genes from other insects identified by NCBI standard protein BLAST (Madden 2013) were constructed using MEGA6 (Tamura et al. 2013). The translation into amino acid sequence was performed using ExPaSy Translation tool (http://web.expasy.org/translate/). ProtParam (Gasteiger et al. 2005) was used to analyze the molecular weight, theoretical pl, amino acid composition, atomic composition, and extinction coefficient. Secondary structure, signal peptide cleavage sites, transmembrane helices in proteins, and protein function were predicted using SOPMA (Geourjon and Deleage 1995), SignalP (Petersen et al. 2011), TMHMM (http://www.cbs.dtu.dk/services/TMHMM/), and ProtFun 2.2 Server (http://www.cbs.dtu.dk/services/ProtFun/).

Construction of pET28a-OfGST Expression Vector

According to the obtained GST gene sequence, the primers OfGST-F-s (containing an NdeI restriction site) and OfGST-F-as (containing a HindIII restriction site) were designed to clone the target gene. The PCR product was digested with NdeI and HindIII and ligated into the expression vector pET28a digested with the same restriction enzymes, to obtain pET28a-OfGST vector.
The recombination expression vector of pET28a was used for transformation of different vectors (E. coli DH5α).

| Bacterial strains | Purpose |
|-------------------|---------|
| E. coli DH5α       | Used for transformation of different vectors |
| E. coli BL21 (DE3) | Used for protein expression |
| E. coli HT115(DE3) | Used for dsRNA production |
| Vectors           |         |
| pMD18-T           | Used for general cloning and sequencing |
| pET28a            | Used for construction of vector pET28a-OfGST |
| pET28a-OfGST      | Used to express OfGST1 gene |
| htlL4440-OfGST    | Used for dsRNA production |

**Primer:** Table 1

| Primers (5’−→3’) | Sequence information | Purpose |
|------------------|----------------------|---------|
| Of-GST-JB-P1     | CCCYCARCACAGTSCGYAC  | Degenerate primers, and PCR size is ~ 340 bp |
| Of-GST-JB-P2     | TAYTTCTGRCYYCTCNAGGA | Detergent complete GST gene, and PCR size is 705 bp |
| Of-GST-F-s       | GCCATATGTGA TCGACGTCTTAC | RACE specific primers |
| Of-GST-F-as      | CGAAAGCTTCACT CCGTCTTGAGCCTTG | Used for construction of RNAi vector htlL4440-OfGST, PCR size is 320 bp |
| GSP1             | GTGGGAACGTGT TGTGAGG  |                   |
| GSP2             | GCTCTAACGTTC CTGGAACCC |                   |
| OfGST-RNAi320-P1 | TCGCTGAGGCAGC TACTTTTATCCCAA |                   |
| OfGST-RNAi320-P2 | GAGAAGCTTCTGCT TGGCTTTAGCTG |                   |
| M13-47           | GCGACCTTTATCCCATCACAC |                   |
| RV-M             | GAGCGGATAACCAA TTTCACACAGG |                   |

The italic sequences were restriction sites; bond and italic letters were protective bases.

### Protein Purification, Antibody Preparation, and Detection

The recombinant expression vector of pET28a-OfGST was transformed into E. coli BL21(DE3) by calcium chloride heat shock method and selected on kanamycin agar plate (Chang et al. 2017). A kanamycin resistant colony was inoculated into LB media containing 100 µg/ml kanamycin and cultivated overnight at 37°C. The following day, 500 µl of the culture was inoculated into 1 liter LB media containing 100 µg/ml kanamycin. When the culture OD600 reached to 0.6~0.8, IPTG was added into the culture to a final concentration of 0.1 mM and then cultivated for another 5 h at 25°C. The cells were gently centrifuged and resuspended in PBS solution before lysis by an ultrasonic homogenizer on ice. After centrifugation at 12,000 × g for 20 min, His-tagged GST was purified with His SpinTrap (GE Healthcare, Pittsburgh, PA). PBS solutions containing imidazole (20, 40, 60, 80, 100, 150, 200, 250, 300, 350, 400, and 500 mM/liter; pH 7.4) were used for gradient elution. The purified protein was kept at −80°C for rabbit polyclonal antibody preparation. SDS-PAGE and western blotting were performed according to the approach by Zhang et al. (Zhang et al. 2015b). Protein concentration was determined by BCA protein assay (Thermo Fisher Scientific, Waltham, MA).

Two New Zealand white rabbits (6 wk old, female, 3 kg) were purchased from BaiQi Biotechnology (Suzhou, China) and used for preparation of rabbit polyclonal antibody. Rabbit serum against GST gene, and PCR size is 320 bp OfGST-RNAi320-P2 was collected after feeding 6, 12, 24, and 48 h according to the approach by Zhang et al. (Zhang et al. 2011). Thirty Asian corn borer larvae of the third instar larvae were randomly chosen and fed with artificial feed (control treatment) (Zhang et al. 2011). Another thirty larvae were fed with artificial feed containing 1 mg/g DIMBOA. Total protein from larvae midgut was extracted after feeding 6, 12, 24, and 48 h according to the approach by Zhang et al. (Zhang et al. 2015b). Western immunoblotting was also used to detect expression of the OfGST1 protein (Zhang et al. 2015b). Total protein extracted from Asian corn borer midgut was diluted 1:500 was used as primary antibody and hybridized with secondary goat anti-rabbit antibody and anti-β-actin.

### Construction of dsRNA Expression Vector

htL4440-OfGST

Based on interference from the cloned GST1 gene, a 320 bp fragment containing very low homologous sequence with GST genes in other insects was chosen to construct a dsRNA expression vector. Primers OfGST-RNAi320-P1 (containing a PstI site) and (pH 9.6), 100 µl per well, at 4°C overnight and then were washed two times with washing buffer. Next, the coated wells were blocked with 200 µl blocking buffer (1% BSA) for 2 h at 37°C, washed one time with washing buffer, followed by incubation with GST antibodies with serial dilutions of 1:2; normal serum without immunization as negative control (diluted 200 times); PBS solution was used as the blank control. After incubation for 1 h at 37°C, the wells were washed three times with washing buffer and incubated with 100 µl HRP-conjugated goat-anti-rabbit IgG (dilution 1:20,000, Santa Cruz Biotechnology, Santa Cruz, CA) for 1 h at 37°C. Finally, the wells were washed three times with washing buffer, and then incubated with 100 µl substrate for 5~15 min at 37°C. Then, color development was stopped by adding 50 µl stop solution (2M H2SO4) and absorbance was measured at 500 nm using a microplate reader.

**Titration:**

**Titration:**
OOfGST-RNAi320-P2 (containing a HindIII site) were designed to amplify the target fragment. After digestion with restriction enzymes, the PCR product, L4440 and L4440 vector were ligated. The expression vector, hL4440-OOfGST, was transformed into E. coli HT115(DE3). IPTG (final concentration 0.4 mM) was used to induce the production of dsRNA. Vector construction was checked by digestion with ApaLI and by production of dsRNA.

Laval Feeding Assay
To test the effects of GST1 dsRNA on OOfGST1 expression and phenotypic effects of its potential silencing in insects, four treatment groups of 30 third instar larvae were fed a diet including ddH2O, IPTG-induced E. coli containing hL4440-OOfGST or the empty vector L4440 and/or 1mg/g DIMBOA mixed into the artificial feed. The control group was fed the artificial feed mixed with sterilized ddH2O. These groups were labeled ddH2O (control), hL4440 (RNA from cells with L4440 only), DIMBOA (DIMBOA only), hL4440+DIMBOA (RNA from cells with L4440 and DIMBOA), and hL4440-OOfGST+DIMBOA (RNA from cells with hL4440-OOfGST and DIMBOA). E. coli containing hL4440-OOfGST or empty vector L4440 was centrifuged at 12,000 rpm for 10 min and diluted with sterilized water (200:1) and then added to the diet at a final concentration of 3 x 10^7 cells/ml. The corresponding amount of dsRNA was not determined. The feed was refreshed and mortality rates were tracked daily for 6 d. The whole experiment was repeated three times.

Results
Total RNA Extraction, cDNA Synthesis, and Complete GST Gene Cloning by 5’ RACE and 3’ RACE
Total RNA was extracted from Asian corn borer and converted to cDNA. The PCR product was about 300 bp in length on a 1% agarose gel (Fig. 1A) and this corresponded with the expected size. The PCR band was cut and purified from the gel and then ligated with vector pMD18-T and positive clones were used for sequencing. The sequencing result indicated that the conservative GST gene was 341 bp in length. Compared this conservative GST sequence with GST-C superfamily: 78% homology with Micromelalopha troglodyte, 74% homology with H. armigera, and 67% homology with B. mori.

5’3’ RACE PCRs both yielded ~ 300 bp PCR products (Fig. 1B and C). Both PCR products were gel extracted and ligated into vector pMD18-T for sequencing. After assembling the sequencing results, a complete GST gene was obtained (Fig. 2), named OOfGST1 and submitted to NCBI GenBank (accession no. JF793681). The whole length was 887 bp, including a 705 bp complete ORF encoding a 234 amino acid peptide. The length of the 5’ UTR was 89 bp, and that of the 3’ UTR was 93 bp.

Characterization of Asian Corn Borer OOfGST1 Gene
The complete GST gene sequence of Asian corn borer was compared with eight other available insect GST genes from GenBank to construct a phylogenetic tree (Fig. 3). OOfGST1 (JF793681), HaGST (ABK40535), MtGST3 (ACT98684), and BmGSTd3 (NP_001037546) form a very stable cluster with 100% bootstrap (Fig. 3). It is possible that OOfGST1 from Asian corn borer is the orthologous gene of these three insects. Its class is unclear at this time. Predicted physical and chemical properties of the OOfGST1 protein are summarized in Supp. Fig. 1.

The cellular roles of OOfGST may include central intermediary metabolism, energy metabolism, signal transduction, and stress response, among other functions (Supp. Table 1).

Construction of pET28a-OOfGST Expression Vector
Using the full cloned OOfGST gene, vector pET28a-OOfGST was constructed and verified by colony PCR and restriction digestion (Fig. 4A and B). Accumulation of soluble OOfGST1 protein was detectable in homogenates derived from E. coli cells containing pET28a-OOfGST after induction with IPTG (Fig. 4C). The protein was not over-expressed in homogenates from cells with the empty pET28a vector. The OOfGST1 protein was purified by affinity chromatography using His SpinTrap (Fig. 4D).

OOfGST1 was purified to a protein concentration of 0.95 mg/ml, sufficient for preparation of an immune antibody. The ELISA results established the titers of the antibody (Table 2). With serial twofold dilutions of GST antibodies, the optical density at 500 nm (OD500) decreased gradually. The results of the negative control (normal serum without immunization diluted 200 times as the negative control) and the blank control (the PBS solution) were 0.153 and 0.022, respectively (Table 2).

Fig. 1. Cloning of the GST gene in O. furnacalis. (A) PCR product of OOfGST1 conserved fragment; (B) PCR result of OOfGST1 gene by 5’ RACE; (C) PCR result of OOfGST1 gene by 3’ RACE. Lane M: DL2000 DNA marker; lane 1: conserved OOfGST1 PCR fragment; lane 2: 5’ RACE PCR fragment; lane 3: 3’ RACE PCR fragment.
Western Blotting Confirmation of Asian Corn Borer OfGST1 Expression in Response to DIMBOA

Asian corn borer fed DIMBOA had increased accumulation of OfGST1. This accumulation increased over time, reaching the highest detected amount at 24 h (Fig. 5).

Construction of htL4440-OfGST and dsRNA Induction Expression

The vector htL4440-OfGST was verified with ApaLI digestion (Fig. 6A). Total RNA from E. coli containing the empty L4440 vector and htL4440-OfGST revealed that only the latter had a detectable accumulation of the ~320 bp dsRNA molecules, the expected size of the dsRNA molecule (Fig. 6B).

Larval Feeding Assay (RNAi Experiment)

Asian corn borer larvae in the control group had normal development while those in the treatment groups had abnormal development (Fig. 7A). Mortality rates increased modestly with the addition of DIMBOA and total dsRNA from htL4440-OfGST. A large increase (over 50%) in mortality was observed when Asian corn borer was fed both the OfGST1 dsRNA and DIMBOA (Fig. 7B).
Discussion

Insect herbivores are largely controlled by plant’s innate defense systems and in farmed crops by artificial chemical biocides. Evolved resistance is a constant risk for all employed insecticides, especially those used in large quantities against quickly reproducing targets. Resistance is observed in both intended and non-intended targets (Blanco et al. 2016, Reid and McKenzie 2016). In addition, chemical pesticides can contribute a troubling amount of persistent pollution (Li et al. 2017, Mansouri et al. 2017). Considering these problems, it is important to continuously develop new and more environmentally benign solutions to control agricultural pests. In recent years, bioengineering bacteria to prevent agricultural diseases and pests has become a research hotspot (Yin et al. 2009, Xue et al. 2012, Zhang et al. 2013). In this study, we cloned a GST gene from the midgut of Asian corn borer. This gene, OfGST1, encodes a stable and hydrophilic protein based on bioinformatics analyses. There may be at least 19 GST genes in Asian corn borer (Cui et al. 2017). Based BLAST and phylogenetic analysis, OfGST1 is closest to uncharacterized

Table 2. ELISA analysis of polyclonal antibody

| Dilution | OD 500 |          |          |          |          |          |
|----------|-------|----------|----------|----------|----------|----------|
| 200      | 1.193 ± 0.002 | 1.378 ± 0.001 | 1.541 ± 0.001 | 1.602 ± 0.001 | 1.655 ± 0.001 | 1.707 ± 0.001 |
| 400      | 1.241 ± 0.003 | 1.317 ± 0.001 | 1.451 ± 0.001 | 1.502 ± 0.001 | 1.554 ± 0.001 | 1.605 ± 0.001 |
| 800      | 1.241 ± 0.003 | 1.317 ± 0.001 | 1.451 ± 0.001 | 1.502 ± 0.001 | 1.554 ± 0.001 | 1.605 ± 0.001 |
| 1,600    | 1.241 ± 0.003 | 1.317 ± 0.001 | 1.451 ± 0.001 | 1.502 ± 0.001 | 1.554 ± 0.001 | 1.605 ± 0.001 |
| 3,200    | 1.241 ± 0.003 | 1.317 ± 0.001 | 1.451 ± 0.001 | 1.502 ± 0.001 | 1.554 ± 0.001 | 1.605 ± 0.001 |
| 6,400    | 1.241 ± 0.003 | 1.317 ± 0.001 | 1.451 ± 0.001 | 1.502 ± 0.001 | 1.554 ± 0.001 | 1.605 ± 0.001 |
| 12,800   | 1.241 ± 0.003 | 1.317 ± 0.001 | 1.451 ± 0.001 | 1.502 ± 0.001 | 1.554 ± 0.001 | 1.605 ± 0.001 |
| 25,600   | 1.241 ± 0.003 | 1.317 ± 0.001 | 1.451 ± 0.001 | 1.502 ± 0.001 | 1.554 ± 0.001 | 1.605 ± 0.001 |
| 51,200   | 1.241 ± 0.003 | 1.317 ± 0.001 | 1.451 ± 0.001 | 1.502 ± 0.001 | 1.554 ± 0.001 | 1.605 ± 0.001 |
| 128,000  | 1.241 ± 0.003 | 1.317 ± 0.001 | 1.451 ± 0.001 | 1.502 ± 0.001 | 1.554 ± 0.001 | 1.605 ± 0.001 |

Each result represented the mean of the three parallel titrations. Normal serum without immunization diluted 200 times was used as the negative control; PBS solution was used as the blank control.
GST genes from other moths. The class of the cloned gene is unclear at this time. DIMBOA is a maize defense compound effective against different insects, fungi, and bacteria. When the compound is included in their diet, Asian corn borer has increased accumulation of OfGST1, suggesting that this protein is involved in the metabolic detoxification of DIMBOA. Ingestion of dsRNA targeting the OfGST1 gene was associated with increased toxicity of DIMBOA in Asian corn borer. This observation suggests that OfGST1 is involved in the detoxification of DIMBOA (Yan et al. 1995).

Asian corn borers are one of the most important crop pests, capable of causing great losses to corn (Nafus and Schreiner 1991). In previous studies, we analyzed the transcriptomics and proteomics of methyl jasmonate-induced defenses in maize leaves against Asian corn borer and found that GSTs were associated with responses to biotic and abiotic stresses (Yang et al. 2015, Zhang et al. 2015b). In this study, using RT-PCR and RACE technology, the complete GST gene from the midgut of Asian corn borer were obtained. Western blotting and RNAi were used to show the possible involvement of OfGST1 in the metabolic detoxification of DIMBOA in Asian corn borer.

Feeding insects with dsRNA and microRNA molecules for RNAi have emerged as laboratory methods that are easy, low-cost, and suitable for long-time observations of gene functions. This method is also used for pest control in agricultural settings. Baum et al. reported the use of specific dsRNA mixed with an artificial diet and fed to the western corn rootworm Diabrotica virgifera as a control method (Baum et al. 2007). Li et al. investigated root dsRNA soaking method to control stem-borers (Li et al. 2015). These results suggest that the RNAi pathway can be exploited to control pests in maize and cotton crops (Baum et al. 2007). Feeding cotton bollworm larvae with plant material expressing dsRNA specific to CYP6AE1a and GST1 significantly delayed larval growth, indicating that engineering crop plants to produce the dsRNA would be a viable idea and would not require exogenous application of dsRNA or microRNA (Mao et al. 2007). In this study, the efficacy of the RNAi method resulted in up to 54% mortality of Asian corn borer third instar larvae simultaneously fed DIMBOA. As most GSTs classes in Asian corn borer may have similar roles in detoxification (Cui et al. 2017), further investigation into if this dsRNA derived from Asian corn borer midgut is also effective to other GSTs classes may greatly contribute to the wide application of RNAi. Generally, Lepidoptera insects are not very sensitive to RNAi, limiting study into RNAi as a control strategy of agricultural pests in this order (Terenius et al. 2011). The development of new RNAi strategies may improve or overcome these limitations. Knock-out mutations of genes contributing to RNAi insensitivity could significantly enhance RNAi efficiency (Guan et al. 2018a,b). To the best of our knowledge, RNAi efforts have not been reported to fail specifically in Asian corn borer. We may be able to use Asian corn borer to investigate the mechanisms underlying RNAi inefficiency in lepidopteran insects. Future research will investigate potential RNAi pathway-related genes, providing a more in-depth analysis of various biological parameters (including developmental stage and rearing conditions) of Asian corn borer to determine the insecticidal effect by dsRNA targeting OfGST1 to further develop this strategy for successful field application.

Fig. 5. Western blot analysis of the expression level of OfGST1 protein. Control: normal feeding; 6, 12, 24, and 48h of the OfGST1 protein expression level after feeding prepared food containing 1 mg/g DIMBOA.

Fig. 6. Construction of htL4440-OfGST and dsRNA induction expression. (A) Enzyme digestion verification of the recombinant plasmid htL4440-OfGST. Lane M1: DL2000 DNA marker; lane 1: L4440 vector digested with ApaiI, and the expected sizes were ~1250 bp and ~1550 bp, respectively; lane 2: recombination vector htL4440-OfGST digested with ApaiI, and the expected sizes were ~1250 bp and ~1850 bp, respectively; (B) Total RNA extracted from E. coli containing L4440 vector or htL4440-OfGST vector. Lane M2: DL1,000 DNA marker; lane 1: total RNA extracted from E. coli containing vector L4440; lane 2, total RNA extracted from E. coli containing htL4440-OfGST dsRNA expression vector, and the expected size of dsRNA was ~320 bp.
Conclusion

In this study, we successfully cloned one complete GST gene from *O. furnacalis*. We also successfully constructed a prokaryotic expression vector to over-express His-tagged OfGST1 under IPTG control and an RNAi vector, htL4440-OfGST, to express dsRNA effectively in contributing to control of Asian corn borer via ingestion. Our data indicated that OfGST1 plays a significant part in detoxification of plant defense compounds like DIMBOA. This knowledge can be applied in agricultural settings to improve the effectiveness of insecticides with similar modes of actions. Additionally, our study provided part of a theoretical basis for investigations into interactions of insect herbivores with plant defense compounds and artificial insecticides.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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Fig. 7. The larval feeding experiment (A) and larval mortality rate (B). Asian corn borer larval mortality rate analysis that fed with different diets. DIMBOA: 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one; *denoting significant difference to control (ddH2O and htL4440 treatments, t-test, \( P < 0.05 \)).
