Pesticide Biochemistry and Physiology 156 (2019) 56–62

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Molecular cloning, characterization and functional analysis of *GluCl* from the oriental armyworm, *Mythimna separata* Walker

Jin-da Wang[a,*,1], Li-fei Chen[a,1], Dong-jiang Lin[a], Jia-song Zhang[a], Ji-han Zhao[a], Da Xiao[b], Ran Wang[b,*], Rong Wang[c], San-ji Gao[a]

[a] National Engineering Research Center of Sugarcane, Fujian Agricultural and Forestry University, Fuzhou 350002, China
[b] Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China
[c] College of Forestry, Fujian Agricultural and Forestry University, Fuzhou 350002, China

ARTICLE INFO

Keywords:
Mythimna separata Walker
Glutamate-gated chloride channel
RNAi
Mortality

ABSTRACT

Glutamate-gated chloride channels (GluCls) mediate inhibitory synaptic transmission in invertebrate nervous systems, and only one *GluCl* gene has been found in insects. Therefore, insect *GluCls* are one of the major targets of insecticides including avermectins. In the present study, a 1347 bp full-length cDNA encoding a 449-amino acid protein (named *MsGluCl*, GenBank ID: MK336885) was cloned from the oriental armyworm, *Mythimna separata*, and characterized two alternative splicing variants of *MsGluCl*. The protein shares 76.9–98.6% identity with other insect GluCl isoforms. Spatial and temporal expression analysis revealed that *MsGluCl* was highly expressed in the 3rd instar and adult head. Dietary ingestion of dsMsGluCl significantly reduced the mRNA level of *MsGluCl* and decreased abamectin mortality. Thus, our results reveal that *MsGluCl* could be the molecular target of abamectin and provide the basis for further understanding the resistance mechanism to abamectin in arthropods.

1. Introduction

Glutamate-gated chloride channels (GluCls), a type of neurotransmitter receptor, are members of the cysteine-loop ligand-gated ion channel family. In the nervous system of invertebrates, GluCls play a critical role in inhibitory synaptic transmission (Cleland, 1996; Jones and Sattelle, 2006). There are five subunits in GluCls and each subunit contains an extracellular N-terminal domain including the glutamate-binding site and four transmembrane (TM) α-helices that form a channel domain. To date, GluCl channels have been described only in invertebrates, which have different numbers of orthologous *GluCl* genes (Jones and Sattelle, 2006; Wolstenholme and Rogers, 2005; Dermauw et al., 2012a). In contrast to other invertebrates, insects such as *Drosophila melanogaster*, *Apis mellifera* and *Tribolium castaneum* have only one *GluCl* gene (Jones and Sattelle, 2007; Knipple and Soderlund, 2010). Because GluCls are only found in invertebrates, they are regarded as ideal insecticide targets with high selectivity (Janssen et al., 2007).

Abamectin belong to the class of macrocyclic lactone insecticides, which have nematocidal, acaracidal and insecticidal activity (Lasota and Dybas, 1991) and now are widely used in the agricultural, veterinary and pharmaceutical fields to control pests (Geary, 2005; Copping and Duke, 2010). GluCls together with gamma-amino butyric acid (GABA)-gated chloride channels have been identified as the primary targets of abamectin in arthropods (Duce et al., 1995; Bloomquist, 2003; Hüter, 2011). To date, most of the information about targeting of GluCls by insecticides has come from studies in model nematodes and insects. (Cully et al., 1994; Kane et al., 2000). The mutations in GluCls have been found to increase resistance to abamectin in the diamondback moth *Plutella xylostella* and the two-spotted spider mite *Tetranychus urticae* (Kwon et al., 2010; Dermauw et al., 2012b; Wang et al., 2016; Liu et al., 2014). In addition, studies have reported that the α subunit is important for the action of glutamate and abamectin in insects, but, in *C. elegans*, both α and β subunits are required for the actionproviding strong evidence that avermectins act on one or more subunits of GluCls (Cully et al., 1994).

The oriental armyworm, *Mythimna separata* Walker is a devastating pest of > 300 types of food and industrial crops, such as corn, rice, and sugarcane (Zhang et al., 2012; Jiang et al., 2014; Wang et al., 2018a). In addition, *M. separata* is a migratory pest with outbreaks in specific years and result in substantial economic damage to local crops (Wang et al., 2006; Jiang et al., 2011). The most common and efficient way to

* Corresponding authors.
E-mail addresses: jdwang@fafu.edu.cn (J.-d. Wang), wangran@ipepbaafs.cn (R. Wang).
[1] Both authors contributed equally to this work.

https://doi.org/10.1016/j.pestbp.2019.02.004
Received 23 January 2019; Received in revised form 4 February 2019; Accepted 5 February 2019
Available online 06 February 2019
0048-3575/

control *M. separata* is by applying chemical treatments including abamectin; however, frequent application of insecticides often results in serious environmental problems and the development of insecticide resistance (Song et al., 2017). Characterization and detailed analysis of the full-length *GluCl* cDNA is fundamental for understanding the molecular mechanism underlying insecticide resistance. While no significant abamectin resistance *M. separata* population was found in the field, studies need to done before resistance occurred. Therefore, to fill the gap in knowledge about the mechanism of insecticide resistance in *M. separata*, we investigated the relationship between *MsGluCl* and susceptibility to abamectin. We cloned and characterized a full-length *GluCl* cDNA (named *MsGluCl*) from *M. separata* and profiled the mRNA expression pattern of *MsGluCl*. In order to explore the molecular targets of abamectin in *M. separate*, we studied the influence of RNAi-mediated knockdown effect on *MsGluCl* expression and investigated the susceptibility of *M. separata* to abamectin after RNAi.

2. Materials and methods

2.1. Insects

The oriental armyworm *M. separata* was originally purchased from Henan Jiyuan Baiyun Industry Company (Jiyuan, Henan, China), and the colony was maintained in the laboratory for 10 years without exposure to insecticides. The insects were kept on an artificial diet under laboratory conditions at 26 ± 1 °C, with 70% relative humidity and a 16 h:8 h (L:D) photoperiod.

2.2. RNA extraction and cDNA synthesis

Total RNA was extracted from several developmental stages (eggs, 1st to 6th instar larvae, pupae and adults) and from different tissues (integument, gut and head) from 6th-instar larvae with the RNeasy Micro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Then the remaining genomic DNA was removed, and the first-strand cDNA was synthesized from 1 μg total RNA using the Prime Script™ 1st Strand cDNA Synthesis Kit with gDNA Eraser (Perfect Real Time) (TaKaRa, Dalian, China) for reverse transcription-polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR).

2.3. Cloning and sequence analysis

The open reading frame (ORF) of the *GluCl* transcript (obtained from our unpublished transcriptome data) was predicted using the tool ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Then, three specific primers (MsGluCl-1-3) were designed to amply the *GluCl* coding region (Table S1) from *M. separata*, the clones were sent to Biosune Co. ltd (Fuzhou, China) for sequencing. The amplified sequences were assembled and aligned with DNAMAN (DNAMAN 5.2.2, Lynnon BioSoft). The gene specific primer (MsGluCl) was used for clone the whole ORF and verify assembled sequence (Table S1). The isoelectric point (pI) and molecular weight (MW) of the deduced protein sequences were analyzed using the ExPASy Proteomics Server (http://cn.expasy.org/tools/pi_tool.html). The BioEdit (https://bioedit.software.informer.com) was used for analysis protein similarity. The mature GluCl protein sequences from *M. separata* and other pest species were aligned using ClustalX 1.83, and a phylogenetic tree was constructed in MEGA 6.0 using the neighbor-joining method with 1000 bootstrap resampling. The signal peptide in MsGluCl was predicted using the SignalP 4.1 server (http://www.cbs.dtu.dk/services/SignalP/), and TM domains were predicted using TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/). Putative motifs were predicted using ExPASy ScanProsite (http://prosite.expasy.org/scanprosite/) or by aligning to other published GluCls.

2.4. qRT-PCR analysis of MsGluCl expression profiles

The relative transcription levels of *MsGluCl* at different developmental stages (eggs, 1st to 6th instar larvae, pupae and adults) and in different body parts (integument, gut and head) from 6th-instar larvae were determined using qRT-PCR, which was performed for three biological replicates using gene-specific primers (Table S1) and SYBR Premix EX Taq™ (TaKaRa, Dalian, China) on the ABI 7500 system (Applied Biosystems, CA, USA). Each 20-μl reaction contained 1.0 μl cDNA (200 ng/ul), 10 μl SYBR Premix Ex Taq™, 0.4 μl forward primer (10 μM), 0.4 μl reverse primer (10 μM), 0.4 μl Rox Reference Dye II, and 7.8 μl nuclease free water. The thermal cycling conditions were: 95 °C for 30 s, 40 cycles of 95 °C for 5 s, 62 °C for 34 s. After the cycling protocol, a melting curve analysis from 60 °C to 95 °C was performed for all reactions to verify the amplification of a single PCR product. The amplification efficiency was estimated using the equation, $E = 10^{-1}/$ slope, where the slope was derived from a plot of cycle threshold (Ct) value *versus* copy number for five serially diluted template concentrations. Quantification of *MsGluCl* transcript level was conducted using the $2_{-\triangle\triangle Ct}$ method (Pfaffl, 2001) and the housekeeping genes *β-actin* and *EF-α* were used as controls to correct for sample-to-sample variation. The expression of *MsGluCl* at different stages and in various body parts was analyzed using one-way ANOVA followed by Tukey's HSD test for multiple comparisons in SPSS 18.0(George and Mallery, 1998).

2.5. Bioassay

Twenty-five 2nd instar larvae were selected and starved for 12 h before being fed an artificial diet containing an 100 μl solution of various doses of abamectin (Shijiazhuang Ruitian Biochemical Co., Ltd., China; 0.1 mg/L, 0.5 mg/L, 1 mg/L, and 5 mg/L) using acetone as the carrier solvent (Song et al., 2017). The criterion to determine death is larval could not move after touching with brush. After 48 h, the mortality rate was recorded, and the corresponding LC_{50} was calculated by Probit analysis in SPSS 18.0. Each concentration was replicated for three times in this experiment.

2.6. RNAi in M. separata

The templates for *in vitro* transcription, a fragment of *MsGluCl* (202 bp) and a fragment of the enhanced green fluorescent protein gene *egfp* (414 bp), were generated by PCR using specific primers (Table S1) conjugated with the T7 RNA polymerase promoter (TAATACGACTCA CTATAGGG). Then double stranded RNAs were synthesized using the T7 Ribomax ™ Express RNAi System (Promega, Madison, WI, USA) as described in the manual.

Thirty late 1st instar larvae were fed on an artificial diet containing 100 μg dsRNA (dsMsGluCl or dsEGFP) in a 24-well plate continuous for 3 days, with fresh dsRNA supplied each day. A diet with the same amount of ddH_2O (100 μl) was used as the control. After 3 days, larvae molted to the 2nd instar stage, and five living larvae in each group (control, *MsGluCl*-dsRNA, and *egfp*-dsRNA) were extracted for total RNA. Then approximately left 20 larvae in each group were used for bioassay test with a dose of 1.7 mg/L (LC_{50}) as described above (section 2.5). Two days after avermectin application, the number of dead larvae were recorded. The experiment was replicated three times.

3. Results

3.1. Analysis of the MsGluCl amino acid sequence and alternative splicing

Three sets of primers were designed to amplify the ORF of *MsGluCl* based on the transcript obtained from *M. separata* transcriptome data. Finally a set of primer was used to amplify the whole ORF and verify the assembled sequence. The assembled ORF consisted of 1347 bp of contiguous sequence and encoded 449 amino acids. This sequence was

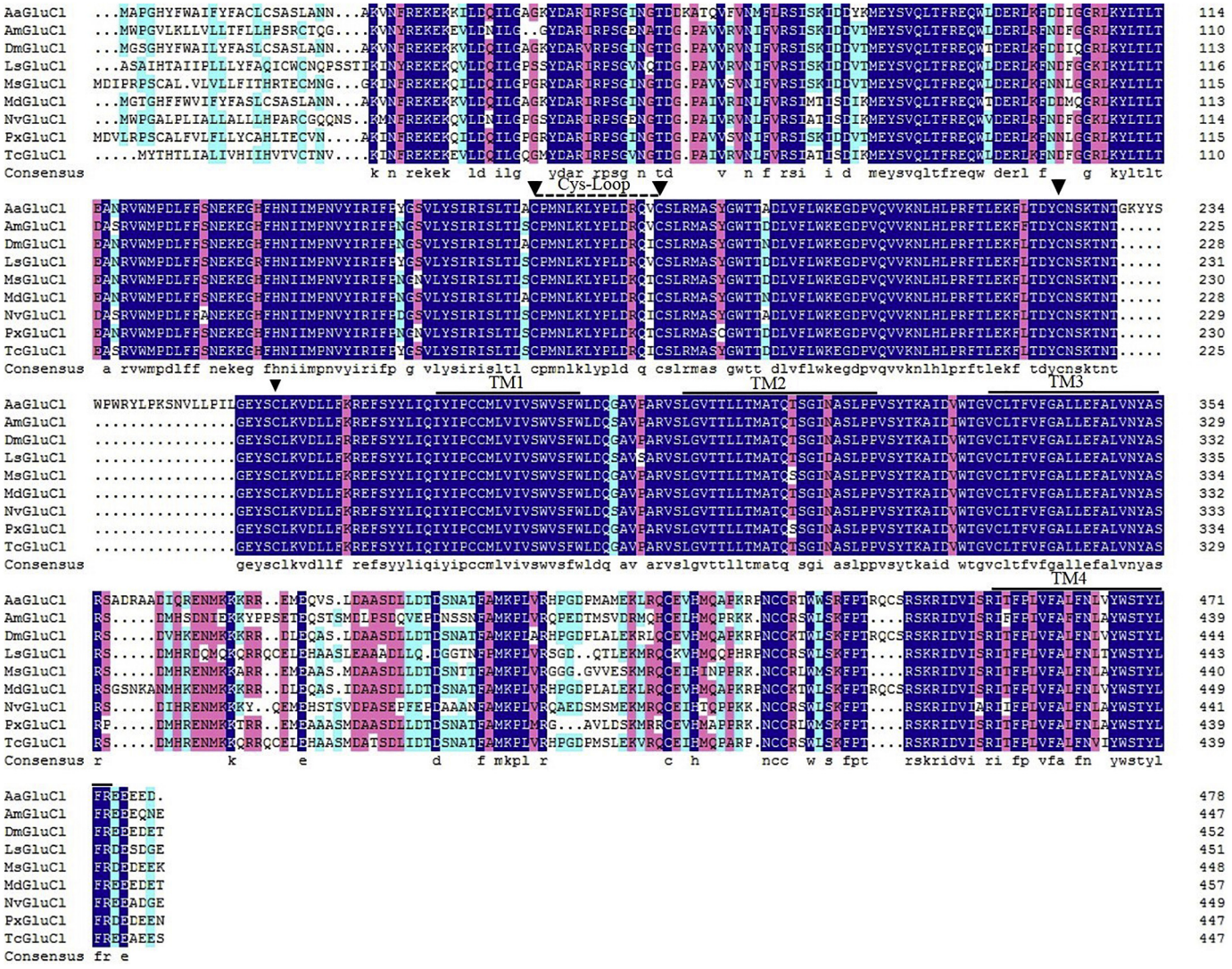

Fig. 1. Alignment of nine insect GluCls. The four transmembrane domains (TM1–4) and four cysteine residues are indicated by black lines and black triangles, respectively. The Cys-loop, the signature domain of neurotransmitter-gated ion-channels, is indicated by a dotted line.

submitted to NCBI under GenBank ID: MK336885.

The predicted MsGluCl protein has a MW of ~51.3 kDa and a pI of 8.71. The MsGluCl amino acid sequence was analyzed for putative regulatory domains. Alignments of insect GluCl proteins showed that there were four typical hydrophobic TM domains in the COOH-terminal region of MsGluCl at positions 253–269 (TM1), 281–302 (TM2), 315–334 (TM3), and 422–442 (TM4) (Fig. 1). In addition, four cysteine residues (C1–C4) in the extracellular domain were found. The sequence motif, CPMNLKLYPLDKQTC, which was identified between C1 and C2 and constitutes part of the neurotransmitter-gated ion-channel signature domain, the Cys-loop, was highly conserved in MsGluCl (165–179) and eight other insect GluCls (Fig. 1).

Alignment of the sequences of multiple cDNA clones revealed one alternative splicing site in the *MsGluCl* transcript, which is predicted to result in the deletion of amino acid residues 372–385 (Fig. 2).

3.2. Phylogenetic relationships of insect GluCl family members

The amino acid sequence identities between GluCl proteins from several orders of insects are shown in Table 1. MsGluCl shared the greatest identity with GluCls from the lepidopteran species *Helicoverpa armigera* (HaGluCl, 98.6%) and *P. xylostella* GluCl (PxGluCl, 93.7%), and also shared a high level of identity with the GluCls from *Tribolium castaneum* (82.3%), *D. melanogaster* (81.3%), and *Laodelphax striatellus* (80.7%). MsGluCl shared the lowest identity with GluCl from *Aedes aegypti* (76.9%).

In a phylogenetic tree of GluCl proteins from 20 species, the insect GluCls were separated from the nematode GluCls (Fig. 3). MsGluCl was clustered with GluCls from the four other lepidoptera species, and GluCls from other species in the same order, such as Diptera, Lepidoptera, Coleoptera, Hymenoptera and Hemiptera, were grouped together.

3.3. Spatial and temporal expression of MsGluCl

Analysis of expression at different developmental stages revealed that *MsGluCl* was expressed at all life stages; *MsGluCl* was most highly expressed in the egg and 1st and 3rd instars, while the expression level during the 4th instar to pupae stage was much lower than that at the other stages. Compared with the 1st instar, the relative expression level of *MsGluCl* mRNA was 1.06-, 0.21-, 1.15-, 0.16-, 0.05-, 0.08-, 0.14- and 0.24-fold higher in the egg, 2nd, 3rd, 4th, 5th, and 6th instar larvae, pupae, and adults respectively (Fig. 4A). *MsGluCl* was highly expressed in the head, while to our surprise, we could not detect *MsGluCl* expression in intestinal tissue (Fig. 4B).

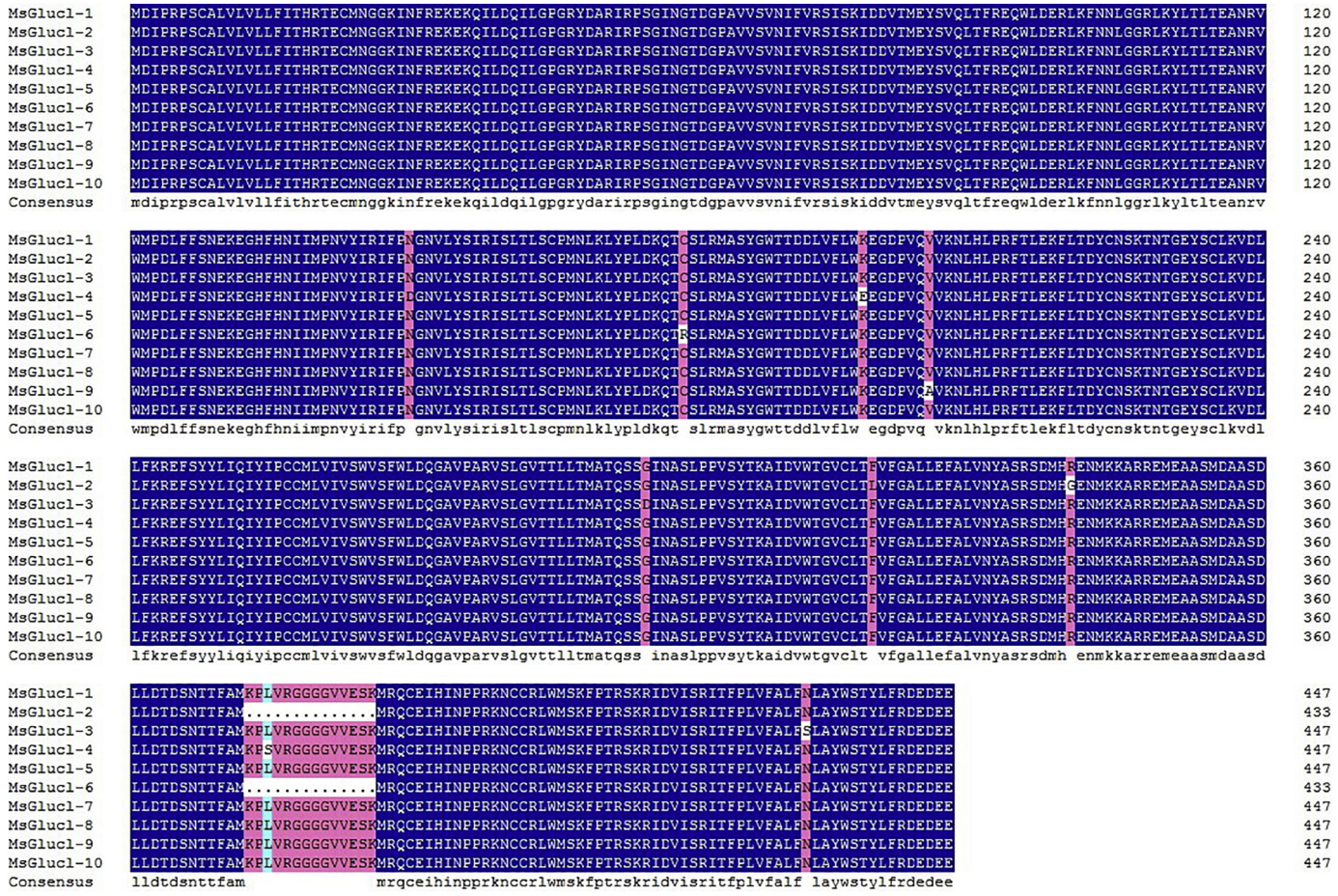

Fig. 2. Sequence alignments of MsGluCl alternative splicing variants.

3.4. Toxicity of abamectin to *M. separata*

The toxicity of abamectin to *M. separata* was determined using the feeding method. Based on bioassay results (Table S2), 1.7 mg/L of abamectin (the LC_{50} dose) was used to treat *M. separata*.

3.5. Effect of dsMsGluCl on MsGluCl expression and abamectin tolerance

After 3 days of continuous ingestion of dsMsGluCl, the late 1st instar molted to the 2nd instar. The levels of *MsGluCl* mRNA in the treated larvae were significantly lower (46.7%) compared with those in corresponding controls. Three days of ingestion of dsRNA killed few larvae. The surviving larvae that had been exposed to CK (ddH_2O), dsEGFP, or dsMsGluCl diets were used for further bioassay experiments. Approximately 20 larvae in each treatment were fed with further abamectin treatment (LC_{50} dose). Larval mortality in both the CK and dsEGFP treatment groups (47.1% and 41.5%, respectively) was much higher than that in the dsMsGluCl treatment group (32.4%, Fig. 5B).

4. Discussion

Avermectin insecticides are widely applied in several crop fields because of its efficient and eco-friendly. In addition, Fluxametamide, another novel wide-spectrum insecticide, which also target to *GluCl* (Asahi et al., 2018). Even though no studies have documented *M. separata* resistance to abamectin, the lack of knowledge about the relationship between the structure and function of *M. separata* GluCl and the potential resistance risk will make it difficult to figure out the

Table 1
Comparison of GluCl sequences from different insects.

	M. sepereta	*H. armigera*	*P. xylostella*	*D. melanogaster*	*M. domestica*	*A. aegypti*	*A. mellifera*	*L. striatellus*	*T. castaneum*
M. sepereta	–	0.986	0.937	0.813	0.798	0.769	0.789	0.807	0.827
H. armigera		–	0.941	0.811	0.796	0.767	0.789	0.807	0.825
P. xylostella			–	0.809	0.794	0.768	0.781	0.803	0.823
D. melanogaster				–	0.943	0.862	0.778	0.786	0.828
M. domestica					–	0.86	0.759	0.769	0.839
A. aegypti						–	0.728	0.731	0.782
A. mellifera							–	0.784	0.806
L. striatellus								–	0.817
T. castaneum									–

The species and their corresponding GenBank IDs are: *Mythimna separata* (MsGluCl), this study; *Laodelphax striatellus* (LsGluCl), AEE39458.1; *Musca domestica* (MdGluCl), BAD16657.1; *Plutella xylostella* (PxGluCl), ACT09139.1; *Tribolium castaneum* (TcGluCl), NP_001107775.1; *Drosophila melanogaster* (DmGluCl), ABG57261.1; *Aedes aegypti* (AaGluCl), XP_021704264.1; *Apis mellifera* (AmGluCl), NP_001071277.1; *Helicoverpa armigera* (HaGluCl), XP_021191010.1.

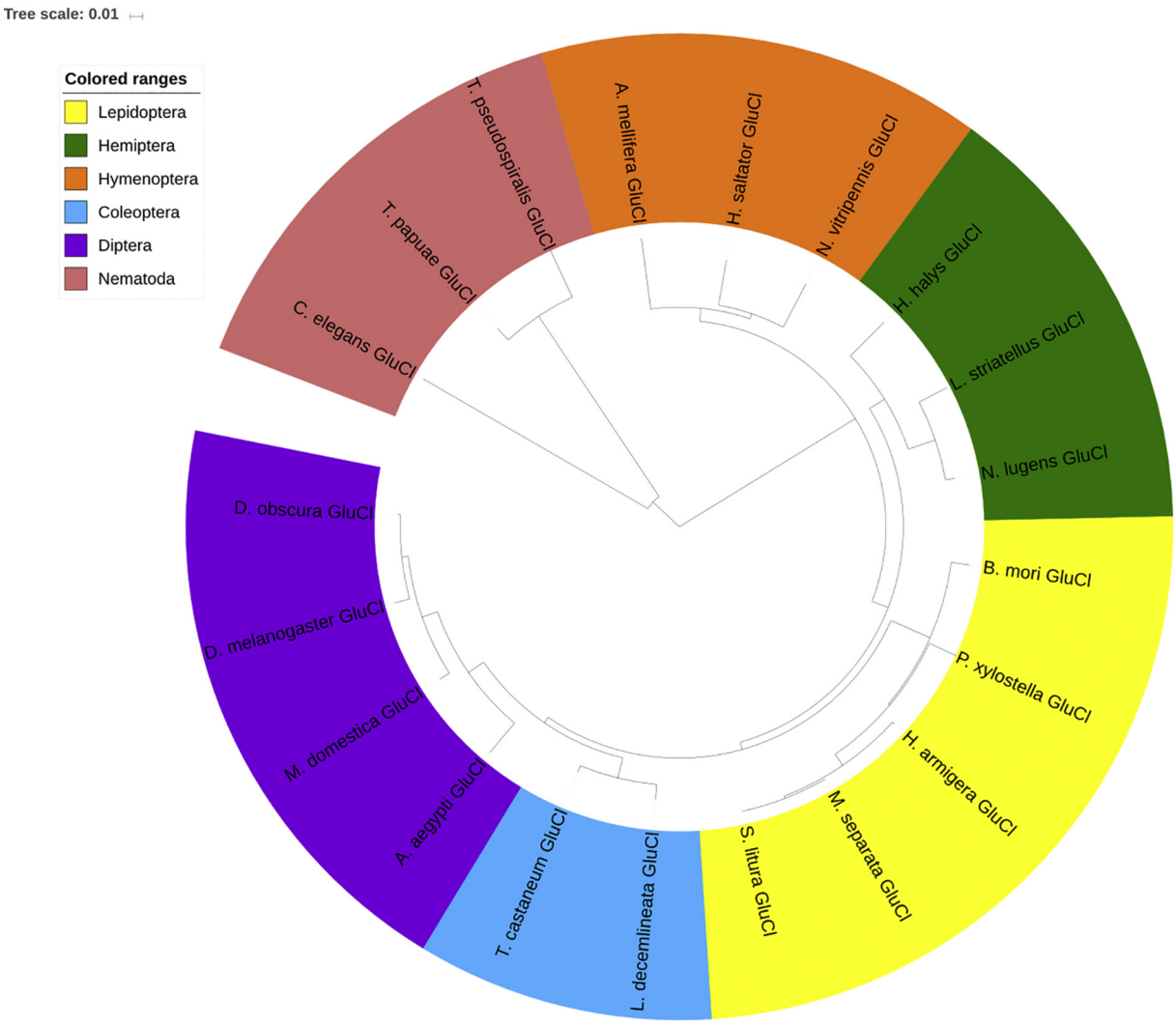

Fig. 3. Phylogenetic tree of the deduced amino acid sequences of MsGluCl and known GluCls from different species.

resistance mechanism when it occurs.

We cloned the full-length *GluCl* coding sequence from *M. separata*, phylogenetic analysis indicated that the MsGluCl amino acid sequence is homologous to that of different insect GluCls. Known domains were also identified in the MsGluCl protein: four cysteine residues within the N terminal region and four transmembrane domains near the C-terminus (Fig. 1). The three TM regions were mostly conserved among insects from different orders including Diptera, Lepidoptera,

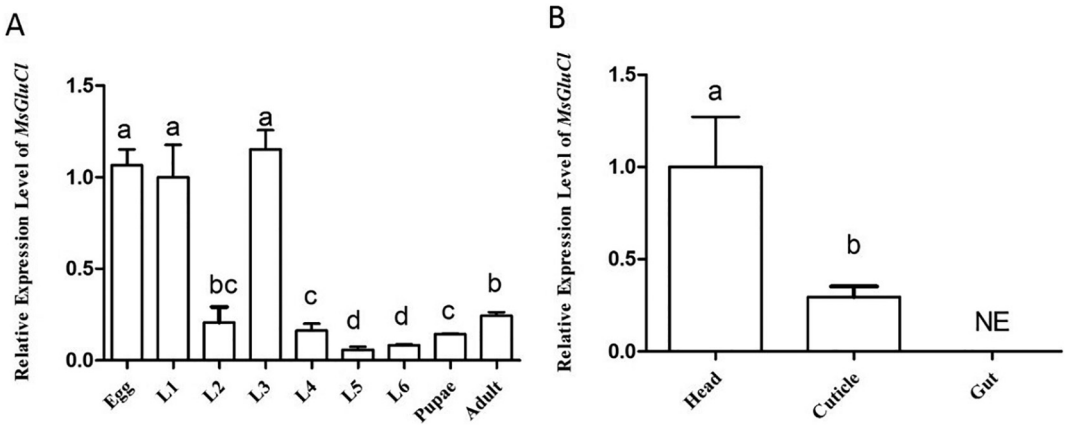

Fig. 4. Spatial and temporal expression of *MsGluCl* mRNA. (A) Relative expression levels of *MsGluCl* mRNA at nine development stages were determined: egg, 1–6 instar nymphs (L1-L4), pupae and adults compared to L1. (B) *MsGluCl* mRNA levels in 6th instar larvae body parts compared to Head. Different lowercase letters (a, b and c) indicate significant differences ($p < 0.05$) based on one-way ANOVA followed by Tukey's HSD test for multiple comparisons. Means ± standard error from three replicates are shown. NE indicates no expression.

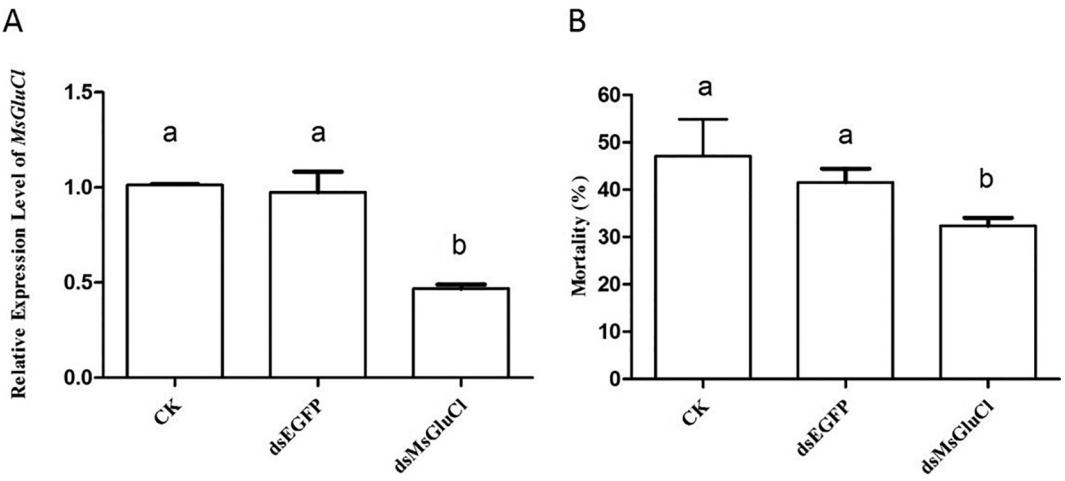

Fig. 5. Effect of the dietary introduction of dsMsGluCl on the relative *MsGluCl* transcript level (A) and mortality of 2nd instar larvae (B). Larvae were continuously fed dsRNA for three days, and mortality was evaluated two days after avermectin or water treatments (CK). Different lowercase letters (a, b and c) indicate significant differences ($p < 0.05$) based on one-way ANOVA followed by Tukey's HSD test for multiple comparisons. Means ± standard error from three replicates are shown.

Coleoptera, Hymenoptera, and Hemiptera, but TM4 was less conserved (Dong et al., 2013; Shi et al., 2014; Meyers et al., 2015). Amino acid sequence alignment showed that MsGluCl shares 76.9–98.6% similarity with other insect GluCl homologs. Phylogenetic analysis showed that insect GluCls are genetically conserved and clearly diverged from the three nematode GluCls, and insect GluCls also clustered together based on insect order (Fig. 3).

GluCls have been found only in invertebrates (Wolstenholme, 2011), hence these proteins are potential targets for the development of pesticides. Several studies have confirmed that mutations GluCl are associated with avermectins resistance. Both electrophysiology and homology modelling studies have demonstrated that three mutations are associated with target-site resistance to abamectin: A309V in *P. xylostella* GluCl (PxGluCl), G323D in *Tetranychus urticae* GluCl1 (TuGluCl1) and G326E in TuGluCl3 (Wang et al., 2016; Wang et al., 2017). In addition, a 36-bp deletion in *P. xylostella* GluCl contributes to target-site resistance to abamectin (Liu et al., 2014). None of the above-mentioned mutations were detected in our *MsGluCl* sequence. Therefore, our work provides valuable data for further studies aimed at detecting the relationship between the mutation and abamectin resistance in the *M. separata*.

Alternative splicing is a key posttranscriptional processing mechanism for generating protein diversity. Two splicing variants were detected in *M. separata*, similar to what has been observed in the insects *D. melanogaster* (Semenov and Pak, 2010), *A. mellifera* (Jones and Sattelle, 2006), *N. vitripennis* (Jones et al., 2010) and *P. xylostella* (Wang et al., 2016). In contrast, three alternative splicing variants of *GluCl* were found in *T. castaneum*, *Laodelphax striatellus* and *Musca domestica* (Hassani et al., 2012; Wu et al., 2017; Eguchi et al., 2010).

To begin to understand physiological roles of GluCl in *M. separata*, we investigated the temporal and spatial expression patterns of *MsGluCl* using qRT-PCR. We found that *MsGluCl* is expressed at all development stages, *MsGluCl* mRNA was the most abundant in the head, in accordance with data from other insects, such as *M. domestica* (Kita et al., 2013), *L. striatellus* (Wu et al., 2017), *B. mori* (Furutani et al., 2014), *B. tabaci* (Wei et al., 2018) and *A. gambiae* (Meyers et al., 2015). It has been reported *GluCl* is expressed in the membranes of the corpus allatum, which synthesizes and releases juvenile hormone and plays an important role in insect metamorphosis (Liu et al., 2005). The *MsGluCl*, which is highly expressed in head tissue, we infer it might play a role in *M. separata* development process. Through the analysis of temporal expression, we found that *MsGluCl* mRNA was highly expressed in the eggs and early larvae except the 2nd instar and was lowly expressed in the L5 and the L6 instars. This expression pattern is similar to, but not exactly the same, as that observed in carmine spider mite *Tetranychus cinnabarinus* (Xu et al., 2017), the silkworm *B. mori* (Furutani et al., 2014) and the small plant hopper *L. striatellus* (Wu et al., 2017). However, not all insect *GluCls* share the same expression pattern as *MsGluCl*. The *GluCl* is highly expressed in adults in *M. domestica* (Kita et al., 2013) and in *B. tabaci* (Wei et al., 2018), and expression of *BtGluCl* was significantly lower in eggs and larvae. Therefore, the temporal expression of *GluCl* varies among insect species.

Theoretically, the change in mRNA expression levels of the target gene should affect the sensitivity of insects to insecticides, and this theory has been confirmed by an increasing number of studies. For example, in the greenbug *Schizaphis graminum*, the amount of acetylcholinesterase mRNA in an organophosphate resistant strain was approximately 1.5-fold higher than that in the susceptible population based on northern blot analysis (Gao and Zhu, 2002). In a laboratory flubendiamide-selected *P. xylostella* strain, the expression level of *PxRyR* was 2.93-fold higher than that in the susceptible strain (Yan et al., 2014). RNAi technology has been widely used to identify or validate insecticide target genes (Kim et al., 2015). Therefore, to confirm the role of *GluCl* in abamectin resistance, we tested sensitivity of *M. separata* larvae to abamectin after targeting *MsGluCl* with dsMsGluCl. Ingestion of dsMsGluCl for 3 days significantly reduced the level of *MsGluCl* mRNA in treated 2nd instars by 53.3%, and greatly decreased abamectin -induced mortality. Our result suggests that *MsGluCl* encodes a functional GluCl that mediates toxicity of abamectin to *M. separata*. Consistent with our finding, knockdown of *GluCl* in *P. xylostella* (Shi et al., 2012), *B. tabaci* (Wei et al., 2018) and *T. cinnabarinus* (Xu et al., 2017) also decreased avermectins -induced mortality.

GluCls play important roles in insect development (Hassani et al., 2012; Kita et al., 2013; Boumghar et al., 2012; Chiang et al., 2002) and have been reported to be involved in the control of locomotion, feeding and sensory input (Wolstenholme, 2011). We previously demonstrated that injection of dsHzGluCl negatively affected egg hatching in corn earworm *Helicoverpa zea* (Wang et al., 2018b); however, we did not observe any negative effects of dsMsGluCl on larval growth during the 3 days of ingestion. Therefore, further work is needed to test the negative effects of dsMsGluCl, and explore the physiological roles of *MsGluCl*.

In the current study, we cloned and characterized the *GluCl* gene from *M. separata* and investigated the function of *GluCl* in abamectin. Further work should be done to reveal the structure and pharmacological characteristics of insect GluCls to provide a basis for understanding the remarkable selectivity of this insecticide towards lepidoptera and the mechanism of resistance.

Acknowledgements

This work was supported by the National Key R&D Program of China (2018YFD201100), the Scientific and Technological Innovation Capacity Construction Special Funds of the Beijing Academy of Agriculture and Forestry Sciences, China (KJCX20180705), the NSFC project (GrantNo. 31601363), the Nature Science Foundation of Fujian (2017J01422) and the Sugar Crop Research System, CARS (CARS-17).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pestbp.2019.02.004.

References

Asahi, M., Kobayashi, M., Kagami, T., Nakahira, K., Furukawa, Y., Ozoe, Y., 2018. Fluxametamide: a novel isoxazoline insecticide that acts via distinctive antagonism of insect ligand-gated chloride channels. Pestic. Biochem. Phys. 151, 67–72.

Bloomquist, J.R., 2003. Chloride channels as tools for developing selective insecticides. Arch. Insect Biochem. 54, 145–156.

Boumghar, K., Couret-Fauvel, T., Garcia, M., Armengaud, C., 2012. Evidence for a role of GABA- and glutamate-gated chloride channels in olfactory memory. Pharmacol. Biochem. Be. 103, 69–75.

Chiang, A.S., Pszczolkowski, M.A., Liu, H.P., Lin, S.C., 2002. Ionotropic glutamate receptors mediate juvenile hormone synthesis in the cockroach, *Diploptera punctata*. Insect Biochem. Mol. Ec. 32, 669–678.

Cleland, T.A., 1996. Inhibitory glutamate receptor channels. Mol. Neurobiol. 13, 97–136.

Copping, L.G., Duke, S.O., 2010. Natural products that have been used commercially as crop protection agents. Pest Manag. Sci. 63, 524–554.

Cully, D.F., Vassilatis, D.K., Liu, K.K., Paress, P.S., Vanderploeg, L.H.T., Schaeffer, J.M., et al., 1994. Cloning of an avermectin sensitive glutamate-gated chloride channel from *Caenorhabditis elegans*. Nature 371, 707–711.

Dermauw, W., Ilias, A., Riga, M., Tsagkarakou, A., Grbic, M., Tirry, L., Van Leeuwen, T., Vontas, J., 2012a. The cys-loop ligand-gated ion channel gene family of *Tetranychus urticae*: implications for acaricide toxicology and a novel mutation associated with avermectins resistance. Insect Biochem. Mol. 42, 455–465.

Dermauw, W., Ilias, A., Riga, M., Tsagkarakou, A., Tirry, L., Van, L.T., Vontas, J., 2012b. The cys-loop ligand-gated ion channel gene family of *Tetranychus urticae*: implications for acaricide toxicology and a novel mutation associated with avermectins resistance. Insect Biochem. Mol. 42, 455–465.

Dong, Y.X., Chen, Y., Wei, Q., Su, J.Y., Gao, C.F., 2013. Cloning and polymorphism analysis of glutamate-gated chloride channel gene of *Laodelphax striatellus* (Hemiptera: Delphacidae). Fla. Entomol. 96, 1168–1174.

Duce, I.R., Bhandal, N.S., Scott, R.H., Norris, T.M., Clark, J.M., 1995. Effects of ivermectin on γ-aminobutyric acid and glutamate-gated chloride conductance in arthropod skeletal muscle, in: molecular action of insecticides on ion channels. Am. Chem. Soc. 250–263.

Eguchi, Y., Ihara, M., Ochi, E., Shibata, Y., Matsuda, K., Fushiki, S., Sugama, H., Hamasaki, Y., Niwa, H., Wada, M., 2010. Functional characterization of musca glutamate- and GABA-gated chloride channels expressed independently and coexpressed in Xenopus oocytes. Insect Mol. Biol. 15, 773–783.

Furutani, S., Ihara, M., Nishino, Y., Akamatsu, M., Jones, A.K., Sattelle, D.B., Matsuda, K., 2014. Exon 3 splicing and mutagenesis identify residues influencing cell surface density of heterologously expressed silkworm (*Bombyx mori*) glutamate-gated chloride channels. Mol. Pharmacol. 86, 686–695.

Gao, J.R., Zhu, K.Y., 2002. Increased expression of an acetylcholinesterase gene may confer organophosphate resistance in the greenbug, *Schizaphis graminum* (Homoptera: Aphididae). Pestic. Biochem. Physiol. 73, 164–173.

Geary, T.G., 2005. Ivermectin 20 years on: maturation of a wonder drug. Trends Parasitol. 21, 530–532.

George, D., Mallery, P., 1998. SPSS for Windows Step by Step: A Simple Guide and Reference. Allyn Bacon, Inc.

Hassani, E.I., Kacimi, A., Steffen, S., Yan, D., Fabien, D., Gérard, L., Catherine, A., 2012. Identification, localization and function of glutamate-gated chloride channel receptors in the honeybee brain. Eur. J. Neurosci. 36, 2409–2420.

Hüter, O.F., 2011. Use of natural products in the crop protection industry. Phytochem. Rev. 10, 185–194.

Janssen, D., Derst, C., Buckinx, R., Eynden, J.V.D., Rigo, J.M., Kerkhove, E.V., 2007. Dorsal unpaired median neurons of *Locusta migratoria* express Ivermectin- and fipronil-sensitive glutamate-gated chloride channels. J. Neurophysiol. 97, 2642–2650.

Jiang, X., Luo, L., Zhang, L., Sappington, T.W., Hu, Y., 2011. Regulation of migration in *Mythimna separata* (Walker) in China: a review integrating environmental, physiological, hormonal, genetic, and molecular factors. Environ. Entomol. 40, 516–533.

Jiang, Y.Y., Chun-Guang, L.I., Zeng, J., Liu, J., 2014. Population dynamics of the armyworm in China: a review of the past 60 years' research. Chin. J. Appl. Entomol. 51, 890–898.

Jones, A.K., Sattelle, D.B., 2006. The cys-loop ligand-gatedion channel superfamily of the honeybee, *Apis mellifera*. Invertebr. Neurosci. 6, 123–132.

Jones, A.K., Sattelle, D.B., 2007. The cys-loop ligand-gatedion channel gene superfamily of the red flour beetle, *Tribolium castaneum*. BMC Genomics 8, 327.

Jones, A.K., Bera, A.N., Lees, K., Sattelle, D.B., 2010. The cys-loop ligand-gated ion channel gene superfamily of the parasitoid wasp, *Nasonia vitripennis*. Heredity 104, 247–259.

Kane, N.S., Hirschberg, B., Qian, S., Hunt, D., Thomas, B., Brochu, R., Ludmerer, S.W., Zheng, Y., Smith, M., Arena, J.P., 2000. Drug-resistant drosophila indicate glutamate-gated chloride channels are targets for the antiparasitics nodulisporic acid and ivermectin. P. Natl. Acad. Sci. USA 97, 13949–13954.

Kim, Y.H., Issa, M.S., Cooper, A.M., Zhu, K.Y., 2015. RNA interference: applications and advances in insect toxicology and insect pest management. Pestic. Biochem. Physiol. 120, 109–117.

Kita, T., Ozoe, F., Azuma, M., Ozoe, Y., 2013. Differential distribution of glutamate- and GABA-gated chloride channels in the housefly *Musca domestica*. J. Insect Physiol. 59, 887–893.

Knipple, D.C., Soderlund, D.M., 2010. The ligand-gated chloride channel gene family of *Drosophila melanogaster*. Pestic. Biochem. Physiol. 97, 140–148.

Kwon, D.H., Yoon, K.S., Clark, J.M., Lee, S.H., 2010. A point mutation in a glutamate-gated chloride channel confers avermectins resistance in the two-spotted spider mite, *Tetranychus urticae Koch*. Insect Biochem. Mol. 19, 583–591.

Lasota, J.A., Dybas, R.A., 1991. Avermectins, a novel class of compounds: implications for use in arthropod pest control. Annu. Rev. Entomol. 36, 91–117.

Liu, H.P., Lin, S.C., Lin, C.Y., Yeh, S.R., Chiang, A.S., 2005. Glutamate-gated chloride channels inhibit juvenile hormone biosynthesis in the cockroach, *Diploptera punctata*. Insect Biochem. Mol. 35, 1260–1268.

Liu, F., Shi, X., Liang, Y., Wu, Q., Xu, B., Xie, W., Wang, S., Zhang, Y., Liu, N., 2014. A 36-bp deletion in the alpha subunit of glutamate-gated chloride channel contributes to avermectins resistance in *Plutella xylostella*. Entomol. Exp. Appl. 153, 85–92.

Meyers, J.I., Gray, M., Kuklinski, W., Johnson, L.B., Snow, C.D., Black, W.C., Partin, K.M., Foy, B.D., 2015. Characterization of the target of ivermectin, the glutamate-gated chloride channel, from *Anopheles gambiae*. J. Exp. Biol. 218, 1478–1486.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RTPCR. Nucleic Acids Res. 29, 45.

Semenov, E.P., Pak, W.L., 2010. Diversification of drosophila chloride channel gene by multiple posttranscriptional mRNA modifications. J. Neurochem. 72, 66–72.

Shi, X.Z., Guo, Z.J., Zhu, X., Wang, S.L., Bao-Yun, X.U., Xie, W., Zhang, Y.J., Qing-Jun, W.U., 2012. RNA interference of the inhibitory glutamate receptor in *Plutella xylostella* (Lepidoptera: Plutellidae). Acta Entomol. Sin. 55, 1331–1336.

Shi, M., Dong, S., Li, M.T., Yang, Y.Y., Stanley, D., Chen, X.X., 2014. The endoparasitoid, *Cotesia vestalis*, regulates host physiology by reprogramming the neuropeptide transcriptional network. Sci. Rep. 5, 8173.

Song, Y., Wang, H., Chen, Y., Wang, S., Sun, H., 2017. Cross-resistance and biochemical resistance mechanisms of emamectin benzoate resistant population of *Mythimna separate*. Chin. J. Pestic. Sci. 19, 18–24.

Wang, G., Zhang, Q.W., Ye, Z.H., Luo, L.Z., 2006. The role of nectar plants in severe outbreaks of armyworm *Mythimna separata* (Lepidoptera: Noctuidae) in China. B. Entomol. Res. 96, 445–455.

Wang, X., Wang, R., Yang, Y., Wu, S., O'Reilly, A.O., Wu, Y., 2016. A point mutation in the glutamate-gated chloride channel of *Plutella xylostella* is associated with resistance to avermectins. Insect Mol. Biol. 25, 116–125.

Wang, X., Puinean, A.M., O'Reilly, A.O., Williamson, M.S., Smelt, C.L.C., Millar, N.S., Wu, Y., 2017. Mutations on M3 helix of *Plutella xylostella* glutamate-gated chloride channel confer unequal resistance to avermectins by two different mechanisms. Insect Biochem. Mol. Biol. 86, 50–57.

Wang, J.D., Chen, L.F., Wang, Y.R., Fu, H.Y., Ali, A., Xiao, D., Wang, R., Gao, S.J., 2018a. Silence of ryanodine receptor gene decreases susceptibility to chlorantraniliprole in the oriental armyworm, *Mythimna separata* Walker. Pestic. Biochem. Phys. 148, 34–41.

Wang, J.D., Gu, L., Knipple, D.C., 2018b. Evaluation of some potential target genes and methods for RNAi-mediated pest control of the corn earworm, *Helicoverpa zea*. Pestic. Biochem. Physiol. 149, 67–72.

Wei, P., Che, W., Wang, J., Xiao, D., Wang, R., Luo, C., 2018. RNA interference of glutamate-gated chloride channel decreases avermectins susceptibility in *Bemisia tabaci*. Pestic. Biochem. Physiol. 145, 1–7.

Wolstenholme, A.J., 2011. Glutamate-gated chloride channels. J. Biol. Chem. 287, 40232–40238.

Wolstenholme, A.J., Rogers, A.T., 2005. Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. Parasitology 131 (Suppl), S85–S95.

Wu, S.F., Mu, X.C., Dong, Y.X., Wang, L.X., Wei, Q., Gao, C.F., 2017. Expression pattern and pharmacological characterisation of two novel alternative splice variants of glutamate-gated chloride channel in the small brown planthopper, *Laodelphax striatellus*. Pest Manag. Sci. 73, 590–597.

Xu, Z.F., Wu, Q., Xu, Q., He, L., 2017. Functional analysis reveals glutamate-gated chloride and γ-amino butyric acid channels as targets of avermectins in the carmine spider mite. Toxicol. Sci. 155, 258–269.

Yan, H.H., Xue, C.B., Li, G.Y., Zhao, X.L., Che, X.Z., Wang, L.L., 2014. Flubendiamide resistance and Bi-PASA detection of ryanodine receptor G4946E mutation in the diamondback moth (*Plutella xylostella L.*). Pestic. Biochem. Physiol. 115, 73–77.

Zhang, Y.H., Zhi, Z., Jiang, Y.Y., Zeng, J., Gao, Y.B., Cheng, D.F., 2012. Preliminary analysis of the outbreak of the third-generation armyworm *Mythimna separata* in China in 2012. Plant Prot. 5, 1–8.