MOLECULAR IDENTIFICATION OF A SHORT-TYPE PEPTIDOGLYCAN RECONGNITION PROTEIN, GMPGRP-SC FROM GRAPHOLITHA MOLESTA

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Peptidoglycan recognition protein (PGRP) as an important pattern recognition receptor, which is found in both invertebrates and vertebrates, play an important role in antibacterial immunity, due to its prominent ability in detecting and eliminating the infection pathogen. However, PGRPs mainly have been identified from Drosophila melanogaster and Bombyx mori, and there were few reports on other agricultural insects, epically about their functions and mechanism. In this study, a short - types PGRP gene named as GmPGRP-SC has been identified in the Grapholitha molesta, oriental fruit moth (OFM) based on analysis of the transcription group database of OFM from our laboratory and the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/). The GmPGRP-SC contains a highly conserved PGRP domain and has the closest genetic relationship with the PGRP gene of Leguminivora glycinivorella according to sequence and phylogenetic analyses. The real-time PCR method was used to analyze its expression pattern in the developmental stage of OFM and in different tissues of the larva of OFM. Finally, the relative expression levels of GmPGRP-SC gene in OFM were analyzed after infected by different treatment of Beauveria bassiana. The results showed that the total cDNA of GmPGRP-SC was 3 221 bp, and the coding regions was 2 268 bp, encoding 756 amino acid residues. The expression level of GmPGRP-SC was the highest in pupal stage of OFM, meanwhile in different tissues of OFM, its relative expression was higher in epithelium and hemocyte, while other stage and tissues were relatively low, and with little difference. The expression level of GmPGRP-SC was significance different when the spore suspension of B. bassiana was 10⁵ conidia/µL infected after 48 h. And when the spore suspension of B. bassiana was 10⁷ conidia/uL, the expression level of GmPGRP-SC was also significance different. All these results lay a foundation for the study of the role and functions of GmPGRP-SC in the innate immunity of OFM, and also do contribute to the further study of the molecular interaction between OFM and B. bassiana. The research results can help to find potential target molecules, and provide scientific basis for the development of new biogenic pesticides and the realization of Green Pest Management (GPM).

Key words: oriental fruit moth; innate immunity; Beauveria bassiana; Green Pest Management.

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Introduction. Insects lack the acquired immune system and mainly rely on their own effective innate immune system for defense against the invasion of fungi, bacteria and other pathogens. Insect innate immune response is induced by the specific recognition of common components bearing on the microbial surfaces, known as pathogen associated molecular patterns (PAMPs), by pattern recognition receptors (PRRs) (Kang et al., 1998; Ochiai & Ashida 1999; Werner et al., 2000). Peptidoglycan recognition proteins (PGRPs) are the most important PRRs in insects, which can recognize the Peptidoglycan (PGN) on the surface of pathogenic microorganisms and activated the Toll and IMD pathways.

Then this can trigger the production of antimicrobial peptides and play a crucial role in the innate immunity of insects against microorganisms (Hultmark, 2003; Beutler, 2004; Lu et al., 2020).

PGRPs as an important part of recognition receptor in insects, play an important role in the immune defense signaling pathway (Toll and IMD), which are natural immunity molecules found in insects, mollusks, echinoderms, and vertebrates, but not present in nematodes or plants (Kang et al., 1998; Dziarski & Gupta, 2006; Gerardo et al., 2010), Nowadavs more than 100 kinds of PGRPs in insects and mammals have been identified, PGRPs can be categorized into two types: long (L) types and short (S) types based on their length. At present, there are nearly 100 members of PGRPs family have been identified, 6 long and 6 short types have been found in Bombyx mori, 6 long and 7 short types in Drosophila melanogaster, and 4 long and 4 short types in Anopheles gambiae 4 PGRP genes in humans and musmusculus (Kang et al., 1998; Tanaka et al., 2008). At present, studies on PGRPs mainly focus on Drosophila melanogaster and Bombyx mori, and fewer studies have been reported in other Lepidoptera insects. In our previous study that the transcription analysis of oriental fruit moth (OFM) reduced by Beauveria bassiana, GmPGRP-SC was up-regulated (the transcriptome data will be published later). So, we hypothesize that GmPGRP-SC gene plays an important role in the anti-fungal immunity of OFM.

The Grapholitha molesta, OFM is a fruit pest all around the world except Antarctica (Natale et al., 2003; Kong et al., 2020). At present, the main method for controling OFM in orchards mainly relies on insecticide and mating disruption (Kanga et al., 2003). Due to the damage of larvae is concealed and resistance to chemical insecticides, it is difficult to control it. Even mating interference has been gradually declined (Benelli et al., 2019). Meanwhile with the further enhancement of people's awareness of environmental protection and pollution-free, the development of effective biogenic pesticides presents a broad application prospect. Due to the extensive use of antibiotics, modern medicine is faced with more and more crises of antibiotic-resistant bacteria. The introduction of each new antibiotic is accompanied by the emergence of antibiotic-resistant strains. In contrast, innate immunity provides the host with immediate protection from infection and has maintained its antimicrobial effect for millions of years without the frequent emergence of drugresistant strains (Zhao et al., 2017; Zhang et al., 2019). Then the concept of biological control based on immune system has been put forward gradually, therefore, it has a great significance to study the immune defense response of OFM for biological control in the future. Then GmPGRP-SC gene is a highly conserved pathogen recognition protein, which plays an important role in the resistance of OFM to pathogenic microorganisms, such as B. bassiana. The B. bassiana is a widely used Entomopathogenic fungus and biological control agent against many kinds of including OFM (Saranraj & Jayaprakash, 2017; Sarker et al., 2020). But there was no report on the interaction between B. bassiana and OFM, especially on molecular aspects. PGRPs as the recognition protein plays a key role in pathogen recognition, activation of Toll and IMD pathway,

and regulation of immune response in insects, and study of it is the basic for studying the molecular mechanism of interaction between *B. bassiana* and OFM (Du et al., 2011). The study of *GmPGRP-SC* gene is helpful to elucidate the function of *GmPGRP-SC* protein in the immune system of OFM and reveal the mechanism of the immune system to recognize pathogenic microorganisms. The research results can help to find potential target molecules, improving the control effect of *B. bassiana* and provide scientific basis for the development of new biogenic pesticides and the realization of green pest control.

Materials and methods. Insect rearing and main instrument

Insect rearing. The insects were kindly gifted by Dr. Zhang Huaijiang from the Fruit Tree Institute, Chinese Academy of Agricultural Sciences. Eggs were reared on Fuji fresh apples until the fifth-instar larvae emerged from the apple. The emerged larvae were reared by artificial diet, adults were fed with 10 % honey solution. The whole developmental period was in artificial climate chamber at 26.5 °C, 75 %–80 % relative humidity, a photoperiod of 15 h light and 9 darkness follow the methods of Du et al (2009).

The main reagent and instruments. Cham Q Universal SYBR real-time PCR Master Mix, HiScript® II Reverse Transcriptase, RNAprep Pure Tissue Kit, Taq DNA Polymerase (Vandesompele et al., 2002; Nolan et al., 2006) and other domestic or imported analytical pure reagents were used. The main software and instrument used in this study were DNAMAN, MEGA 7.0, Bio-Rad PCR instrument (Lin & Yao, 2012), Eppendorf 5425 R small high speed refrigerated centrifuge was used (American), ABI QuantStudio5 Q5 (Bustin et al., 2009; Pavsic et al., 2016)

Primer design. The complete sequence of *GmRP-SC1* gene and the conserved region were obtained from the transcriptome analysis data of our laboratory, the primers were designed by DNAMAN, and the synthesis of the primers was completed by Sangon Biotechnology (Shanghai) Co., Ltd (Table 1).

Table 1

Lists of the primer sequences (Pantty et al., 2003)					
Primer name Primer sequence 5'→3'					
PGRPF	TCAAGTGCGGAGTGACCAA				

PGRPF	TCAAGTGCGGAGTGACCAA
PGRPR	ATGCCATCAAGATTGTCGG
EF-1aF	CATCACAGTAAAGGACGGTAAG
EF-1aR	AGAACAAGACCAGAGCATCC

Sequence analysis and evolutionary tree construction. The complete spliced cDNA sequence of *GmPGRP-SC* was analyzed. ORF prediction and protein translation were performed by DNAMAN. Protein physical and chemical properties, structural domain prediction using proteomics ExPASy was done online (http://web.expasy.org/protparam/ (Artimo et al., 2012) with the help of SignalP-5.0 Server (http://www. cbs.dtu.dk/servi- ces/SignalP/ (Armenteros et al., 2019) and SMART (http://smart.emblheidelberg.de/ (lvica & Peer 2017) analysis. NCBI Blast Network Server was used to search the homology of *GmPGRP*-SC gene

amino acid sequence of 16 species of insects, including *Leguminivora glycinivorella* and *Bombyx mori* (Geer et al., 2010). The phylogenetic tree was constructed by Neighborjoining method (Telles et al., 2018), and the phylogenetic relationship was analyzed by using the biological software Mega 7.0. (Sudhir et al., 2016).

The spatio-temporal expression models of GmPGRP-SC gene in OFM. The samples of RNA were extracted at different development stages (first instar larva, second instar larva, third instar larva, fourth instar larva, fifth instar larva, pupa, and adult) and sample of different tissue were extracted from the larvae that second days after fifth-instar larva, including hemocyte (70 to 80 samples were taken from each sample, and the hemolymph was collected at 4000 g/min), fat bodies, epidermis, malpighian tubules and midgut, according to the method of RNAprep Pure Tissue Kit. RNA purity and integrity were checked with Agilent 2100 bioanalyzer (Fischer & Siedler, 2004; Rufer A., 2010).

Real-time fluorescent quantitative PCR. Then the samples were performed to reverse transcription by the kit of HiScript® II Q RT SuperMix for real-time PCR (Soohyun et al., 2011). The first strand of sample cDNA was used as a template, and each sample was repeated for 3 times. PGRPF and PGRPR were used as primers for fluorescence quantitative amplification, and Ef-1 α were used as a housekeeping gene from the reference gene selection experiment (Cao, 2015). The real-time PCR was calculated by relative quantitative method follow the instructions of ChamQ Universal SYBR real-time PCR kit. The instruments were ABI QuantStudio5 Q5 (American) real-time PCR, the reaction conditions were as follows: predenaturation at 95 °C for 30s. 95 °C for 10s. 60 °C for 30 s. a total of 40 cycles, 95 °C for 15 s, 60 °C for 60 s, 95 °C for 15s for recording the dissolution curve (Nolan et al., 2006)

Effect of the expression of GmPGRP-SC in OFM after infected by B. bassiana

B. bassiana culture and conidia suspension preparation. B. bassiana BNCC 111705 was from BeNa Centure Collection, and cultured on potato dextrose (PDA) plates at 28 °C, 95 %

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Fig 1. The cDNA and encoding amino acid sequence of the GmPGRP-SC. (The underlined signal indicates the signal peptide sequence; the double underline indicates the PGRP domain, and the start and stop codon are indicated in the boxes)

humidity in complete darkness. Conidia were collected form the plate incubated for 5–7 days. Then the surface of the mycelium was scraped and filtered with sterile gauze, washed with ddH₂O for third times, counted and adjusted to 1×10^5 conidia/µL, 1×10^7 conidia/µL, 1×10^9 conidia/µL respectively with hemocytometer (Liu et al., 2014). Freshly prepared conidia were used for all experiment.

Sample preparation and treatment by the infection of *B. bassiana*. The fifth instar larvae with consistent growth were selected and used for infection. The treatment groups of insects were soaked in the spore suspensions with different concentrations separately (1×10^5 conidia/µL, 1×10^7 conidia/µL, 1×10^9 conidia/µL) for 10s, and the control groups were used as ddH₂O for the same time. Then the water on the surface of the insects was drained with sterile filter paper, and the insects reared separately in each dactyllethrae with artificial diet. Each assay was repeated three times. After treatment of 24 h, 48 h, 72 h, the samples of treatment groups and control groups were immediately frozen in liquid nitrogen. Total RNA extracted and reverse transcription by the method of 2.4. real-time PCR validation and analysis were the same as 2.5.

Statistical analyses. The relative expression of the gene was calculated by the method of $2^{-\Delta\Delta Ct}$, $-\Delta\Delta Ct=-(\Delta Ct. q-\Delta Ct. ab)$,

- Ct represents the number of cycles the target amplification product under went to reach the set thre shold,

 $-\Delta Ct$ means difference of Ct value between target gene and housekeeping gene

- q means target gene (GmPGRP-SC is in this experiment)

- *ab* means housekeeping gene (*Ef-1* α gene is in this experiment) (Livak et al., 2001).

All the results were analyzed with Student's t-test in SPSS 19.0 software (Wang & Wang, 2011). Significant difference was indicated by *(P < 0.05), **(P < 0.01) respectively. The data was presented as mean \pm standard error (SE).

Results. 3.1. Sequence analysis and phylogenetic tree of *GmPGRP-SC*. The complete sequence of *GmPGRP-SC* gene was obtained by analyzing the data of the transcriptome

data in our laboratory, and it has been submitted to NBCI GenBank. The GenBank accession number is MW773839. The results shows that the full length of the GmPGRP-SC cDNA sequence is 707 bp, the length of the open reading frame (ORF) is 621bp, and it encoded 206 amino acid residues. The predicted signal peptide is located between 1~33 amino acids, the transmembrane region is between 7~26 amino acids. The molecular weight is predicted to 22.75 KD. Total number of negatively charged residues (Asp + Glu) is 15 and total number of positively charged residues (Arg + Lys) is 21. The instability index (II) is computed to be 26.60, and this classifies the protein as stable. The theoretical pl (isoelectric point) is 9.19. The Grand average of hydropathicity (GRAVY) is -0.1.

The domain structure was analyzed by SMART software online. The result shows that the PGRP domain structure is located

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Trichoplusie ni PGRP CGVEPENLTANENIVGHPCLISTESTERREINETREDNF 163	Samia ricini PGRP-A	COVNNEHLDSDANVVGHRELMATDSDERKLYNIIRDEDEW	184
	Trichoplusia ni PGRP	COVER MILTAN MIVGHROLISTESPORKLYNEIRRODMF	102

Fig 2. Multiple sequence alignment of *GmPGRP-SC* with the homologs of other insects based on amino acid sequence (The PGRP and correspond GenBank accession numbers are as follows. *Bombyx mandarina* PGRP: XP_028043866.1; *Bombyx mori* PGRP: NP_001036836.1; *Drosophila melanogaster* PGRP-SC1A: CAD89163.1; *Gileria mellonella* PGRP-SC2: XP_026759339.1; *Helicoverpa armigera* PGRP-A: AHK59818.1; *Leguminivora glycinivorella* PGRP-SC1 AXS59124.1; *Manduca sexta* PGRP-1A: AAO21509.1; *Operophtera brumata* PGRP-SA: KOB63145.1; *Ostrinia furnacalis* PGRP: ABZ81267.1; *Papilio machaon* PGRP: KPJ06010.1; *Papilio xuthus* PGRP: XP_013170473.1; *Papilio xuthus* PGRP-SA: BAM19609.1; *Plutella xylostella* PGRP-1R: QCS60952.1; *Plutella xylostella* PGRP-S2: AUI41055.1; *Samia ricini* PGRP-A: BAF03522.1; *Trichoplusia ni* PGRP: XP_026737257.1, amino acids with 100%, 75%, 50% identity are in black, gray, and white box, respectively.)

between 35~177 amino acids (Fig. 1). Blast search results showed that the amino acid sequence of *GmPGRP-SC* was highly consistent with that of other insects. These features indicated *GmPGRP-SC* belong to the PGRP-S family.

The amino sequences of *GmPGRP-SC* protein and other 16 species PGRPs of insects, including *Bombyx mori* PGRP-S and *Plutella xylostella* PGRP-SC2 multilinked by BLASTX search. The results showed that the amino acid sequence of *GmPGRP-SC* and that of *Leguminivora glycinivorella* PGRP-SC had the highest consistency more than 93 %, and with that of *Papilio machaon* PGRP, *Papilio xuthus* PGRP, *Papilio xuthus* PGRP-SA were more than 79 %. Furthermore the consistency with other 12 insects were more than 50 % (Fig. 2).

An evolutionary phylogenetic tree was constructed with the amino acid sequences of the remaining 16 insect species. The results showed that when it had closer the genetic relationship, the homology with the *GmPGRP-SC* amino acid sequence was higher. For example, the *Leguminivora glycinivorella* PGRP-SC forms a branch with highest homology, and the confidence coefficient is 100. Meanwhile the amino acid homology of *GmPGRP-SC* with insects such as *Glleria mellonella* PGRP-SC2 and *Drosophila melanogaster* PGRP-SC1A are very low, and the genetic relationship is relatively distant, which forms to different branches (Fig. 3).

3.2. The developmental expression pattern of GmPGRP-SC gene in OFM. To characterize the function of GmPGRP-SC, we first analyzed its expression pattern. Samples of OFM at different developmental stages were taken, and Ef-1a was used as the housekeeping gene. The relative expression of GmPGRP-SC gene in different developmental stages of OFM was compared. The real-time PCR result showed that in different developmental stages of OFM GmPGRP-SC gene was all







Fig. 4. The temporal expression level of *GmPGRP-SC* gene (Data in the figure are mean ± SD)

The temporal expression level of GmPGRP-SC gene

Samples	GmPGRP-SC gene Ct	<i>Ef-1α</i> gene Ct	Relative expression level	Average	Standard deviation
	22.466	24.091	1.236111073	1.029029912	0.230511995
first instar larvae	22.578	24.129	1.143529957		
	23.271	24.118	0.707448706		
	21.192	22.732	0.77343976	0.525177734	0.218540028
second Instar	22.005	22.078	0.440215823		
lai vac	22.288	21.679	0.361877618		
	20.093	20.260	0.592918174	0.666356378	0.085862331
third instar larvae	19.971	20.856	0.645391319		
	19.734	20.926	0.760759642		
	21.364	22.583	0.94619112	1.028119914	0.135108167
fourth instar larvae	21.352	22.247	0.954106178		
	21.040	23.046	1.184062445		
	20.979	19.596	0.252950515	0.303104737	0.072546125
fifth instar larvae	20.884	20.150	0.27007543		
	20.368	21.266	0.386288266		
	18.924	23.311	4.402813784	4.27639272	0.54896635
pupal	18.976	21.806	4.246502504		
	19.158	22.093	3.742982119		
	18.787	20.788	4.839693538	0.203418039	0.053216785
adult	21.855	20.660	0.177533198	1.029029912	0.230511995
	21.934	20.661	0.168095488		

Table 3

The relative expression level of GmPGRP-SC gene in different tissues of Grapholitha molestae larva

Samples	GmPGRP-SC gene Ct	<i>Ef-1α</i> gene Ct	Relative expression level	Average	Standard deviation
	18.80292702	19.15328789	1.236111073	0.965881618	0.029836818
head	18.88503456	19.19662666	1.143529957		
	18.87242699	19.35869598	0.707448706		
	19.99529457	23.17064857	0.77343976	10.6278559	3.975745188
epidermis	18.85653114	23.14433479	0.440215823		
	19.30094528	23.14778519	0.361877618		
	29.18466949	21.02428055	0.592918174	0.003568288	0.000828351
fat body	28.85754013	20.95024109	0.645391319		
	28.51244545	21.41017342	0.760759642		
	18.54099274	18.56934738	0.94619112	0.711292984	0.062598954
midgut	18.76748085	18.47967911	0.954106178		
	18.54175377	18.61497879	1.184062445		
	25.99302483	22.90022278	0.252950515	0.090309401	0.004558024
malpighian tubule	26.04649162	23.05208969	0.27007543		
	26.13798714	23.11437225	0.386288266		
hemocyte	18.08861351	21.76060867	4.402813784	9.340752789	0.326496862
	18.18934059	21.8168602	4.246502504		
	18.14170456	21.81087494	3.742982119		

expressed, but with different levels of expression. Its expression level was higher at pupa stage of OFM about 8 times than fifth instars larvae, then in the adult, the relative expression was low, nearly the same as the fifth instars larvae (Tab. 2, Fig. 4).

Differenttissuesfromthelarvaeofthefifthinstaratthesecond day were taken, and *Ef-1* α was used as the housekeeping gene, the relative expression of *GmPGRP-SC* gene in different tissues of OFM was compared. The result showed

that the relative expression of *GmPGRP-SC* gene was significant difference between different tissues of larvae.

3.3. The relative expression level of GmPGRP-SC gene in different tissues of the larva of OFM. The highest expression level was in epidermis and hemocyte, and the level was as high as about 10 times in comparison with other tissues. While, it was barely expressed in fat body tissues, and the expression levels of GmP-GRP-SC gene in other tissues were not the same. (Tab. 3, Fig. 5).



Fig. 5. The relative expression level of *GmPGRP-SC* gene in different tissues of *Grapholitha molesta* larva (Data in the figure are mean ± SD)

3.4. The effect to the expression level of GmPGRP-SC in OFM after infected by B. bassiana. In order to analyses expression changes after immune stimulations, we performed real-time PCR to analyses the transcript level reduced by different treatment of *B. bassiana*. The result showed that the infection of B. bassiana can induced expression of the GmPGRP-SC gene, this was consistent with the results that PGRPs involved in the immune function. The effect on the expression of GmPGRP-SC gene was different when the spore concentration and infected times of B. bassiana were different. When B. bassiana was 10⁵ conidia/µL, after 48 h of infection, the expression of GmPGRP-SC gene was significance between the CK group and Treatment group. But there was no significant difference of the GmPGRP-SC gene between 24 h and 96 h after infected with B. bassiana (shown in Tab. 4, Fig. 6).



Fig. 6. The temporal expression level of *GmPGRP-SC* gene of different time treated with 10⁵ conidia/µL spore suspension of *B. bassiana* (Data in the figure are mean ± SD. The asterisks above bars indicate significance between treatment and the CK determined by the student's t-test, respectively. The same for the following figures) The *B. bassiana* was 10^7 conidia/µL, after 24 h and 48 h, the expression of *GmPGRP-SC* gene was significance between the CK group and treated group. While *B. bassiana* was 10^9 conidia/µL, there was no significant difference between treated and control group, and the expression of *GmPGRP-SC* gene was slightly down-regulated (shown in Tab. 5–6, Fig. 7–8).







Fig. 8. The temporal expression level of *GmPGRP-SC* gene of different time treated with 10⁹ conidia/µL spore suspension of *B. bassiana* (Data in the figure are mean ± SD)

Discussion. Members of the *PGRPs* family, including insects and mammals, are highly conserved. There is a PGRP domain composed of about 165 amino acids at the C-terminal,

The temporal expression level of *GmPGRP-SC* gene of different time treated with 105 conidia/µL spore suspension of *B. bassiana*

Samples	GmPGRP-SC gene Ct	<i>Ef-1α</i> gene Ct	Relative expression level	average	Standard deviation	Student's t test (P) CK and treatment
	21.89202309	21.97925758	1	1	0.140738294	
Ck-24h	21.53461075	22.09150314	1.281125969			
	21.71774483	21.95654106	1.128399756			
	23.12648392	22.68375015	0.693500568	0.680421291	0.026335	0.005268216
Treatment-24h	23.21970367	22.86681175	0.650107053			
	23.11786461	22.59602928	0.697656253			
	22.63484001	20.65943527	1.060431412	1.439278827	0.591494525	
Ck-48h	21.63484001	21.57265854	2.120862824			
	22.53484001	22.10047913	1.136542246			
	19.61118698	22.82375336	8.340906511	8.155285513	0.194446749	4.83226E-05
Treatment-48h	19.64072418	23.00129509	8.171874155			
	19.67987823	22.54035759	7.953075873			
	23.95530319	24.68696785	1.799966838	2.693724964	2.693724964	
Ck-72h	23.07602501	25.15373421	3.31095754			
	23.2326889	25.24067116	2.970250515			
	20.73429298	22.16456795	2.318965356	2.40994224	0.084880236	0.570824228
Treatment-72h	20.63336372	22.13929558	2.48700693			
	20.67047119	21.89069176	2.423854435			

Table 5

The temporal expression level of *GmPGRP-SC* gene of different time treated with 10^7 conidia/µL spore suspension of *B. bassiana*

Samples	GmPGRP-SC gene Ct	<i>Ef-1α</i> gene Ct	Relative expression level	average	Standard deviation	Student's t test (P) CK and treatment
	21.89202309	21.97925758	1	1	0.140738294	
Ck-24h	21.53461075	22.09150314	1.281125969			
	21.71774483	21.95654106	1.128399756			
	22.28517914	22.68375015	6.762721597	6.012059955	1.168196308	0.001996064
Treatment-24h	22.31871605	22.86681175	6.607328401			
	22.82055664	22.59602928	4.666129867			
	22.63484001	20.65943527	1.060431412	1.439278827	0.591494525	
Ck-48h	21.63484001	21.57265854	2.120862824			
	22.53484001	22.10047913	1.136542246			
	20.07825851	22.82375336	6.762721597	4.580062879	0.300287135	0.001204667
Treatment-48h	19.88920212	23.00129509	6.607328401			
	19.98667145	22.54035759	4.666129867			
	23.95530319	24.68696785	1.799966838	2.693724964	2.693724964	
Ck-72h	23.07602501	25.15373421	3.31095754			
	23.2326889	25.24067116	2.970250515			
Treatment-72h	21.23572922	22.16456795	2.27191037	2.315191159	0.300287135	0.529663554
	21.56582069	22.13929558	1.80727299			
	20.90040016	21.89069176	2.866390119			

which plays a crucial role in the recognition of exogenous substances. Different PGRP domains may be the mechanism for distinguishing and identifying different kinds of microorganisms (Blanco et al., 2008). The results of *GmPGRP-SC* gene in our study are consisting with this. The amino acid sequence prediction results shows that *GmPGRP-SC* has a conserved domain structure. This structure also finded in *Helicoverpa armigera*, *Manduca sexta* and *Tribolium castaneum* etc. (Gottar et al., 2002). We explored the evolution and conservation

of *GmPGRP-SC* with other insects. The phylogenetic tree shows that *GmPGRP-SC* has high homology and close genetic relationship with *Leguminivora glycinivorella*. Except when the data is missing, the PGRP sequences reasonably generalize the tree of hypothetical species in each gene family (Wiegmann et al., 2011).

The report about the *Musca domestica* and *Drosophila melanogaster* has the similar result with the gene *MdPGRP-SC*, it is speculated that this gene may play an important role in

The temporal expression level of GmPGRP-SC gene of different time treated
with 10 ⁹ conidia/µL spore suspension of <i>B. bassiana</i>

Samples	GmPGRP-SC gene Ct	<i>Ef-1α</i> gene Ct	Relative expression level	average	Standard deviation	Student's t test (P) CK and treatment
	21.89202309	21.97925758	1	1	0.140738294	
Ck-24h	21.53461075	22.09150314	1.281125969			
	21.71774483	21.95654106	1.128399756			
	21.59008408	22.68375015	1.311036896	1.301804097	0.167356775	0.260578719
Treatment-24h	21.43052864	22.86681175	1.464353353			
	21.80444145	22.59602928	1.13002204			
	22.63484001	20.65943527	1.060431412	1.439278827	0.591494525	
Ck-48h	21.63484001	21.57265854	2.120862824			
	22.53484001	22.10047913	1.136542246			
	23.84972191	22.82375336	0.465275442	0.521271989	0.065653954	0.055704075
Treatment-48h	23.49849129	23.00129509	0.593527824			
	23.73148727	22.54035759	0.505012702			
	23.95530319	24.68696785	1.799966838	2.693724964	2.693724964	
Ck-72h	23.07602501	25.15373421	3.31095754			
	23.2326889	25.24067116	2.970250515			
Treatment-72h	20.19152832	22.16456795	1.990094957	2.228339352	0.357441546	0.406331633
	19.78418732	22.13929558	2.639341944			
	20.14481926	21.89069176	2.055581155			

the pupal stage (Werner, 2000; Gao, 2013). But Bd PGRP-SB1 is highly expressed in 3rd larvae and adults of Bactrocera dorsalis (Zhang et al., 2020). All these three are belong to Diptera, and the developmental expression pattern of PGRP gene in Lepidoptera has been reported in Antherea pernyi, but without significant change (Liu W., 2019). And in other insects of Lepidoptera, there is no more report, whether it is related to the developmental regulation and immunity, it needs to be further studied. The expression level of GmPGRP-SC is greatest in the pupal stage and in epidermis and hemocyte. But in Antherea pernyi the ApPGRP-A, ApPGRP-B, ApPGRP-C have no significant change. Epidermis and hemocyte is related to its immune response, so in these tissues are highly expressed. The result is consisting with that of Antheraea pernyi, and the APPGRP-C gene is expressed in immunerelated groups, such as hemolymph and epidermal (Liu W., 2019). In Bombyx mori, Bm PGRP-S4 is highly expressed mainly in hemolymph, which may be involved in the systemic immune response of Bombyx mori, depending on hemolymph circulation (Yang et al., 2017).

The real time-PCR results showed that the expression of *GmPGRP-SC* gene was up-regulated in different times after infected with 1×10^5 conidia/µL and 1×10^7 conidia/µL of *B. bassiana*. And 1×10^9 conidia/µL may inhibit the normal development of OFM. In *Ostrinia furnacalis*, member of *PGRP* genes was up-regulated when infected by *B. bassiana* with 2×10^5 conidia/µL (Liu et al., 2014). The *MxPGRP-1* in *Manduca sexta* was up-regulate after infected by *Escherichia coli* (Sumathipala & Jiang, 2010). In *Drosophila*, the expression of *DmPGRP-LB*, *DmPGRP-SA*, *DmPGRP-SB1*, *DmPGRP-SC2 and DmPGRP-SD* were strongly up-regulated by *Bacillus subtilis* and purified peptidoglycan (Werner et al., 2000). The mortality became higher as concentration increase when the OFM was infected with different concentration of *B. bassiana* ARP14 (Sarker et al., 2020). These are consistent with the results of our study, but the function of GmPGRP-SC gene in the immune signaling pathway needs to be further studied. In this study, we also observed that when infected with 1×10^9 conidia/µL, the growth and development of OFM were slow. But due to the small number of insects in our experiment, it does not constitute ecological statistics. So, we supposed that possible infection of *B. bassiana* with 1×10^9 conidia/µL, affected the normal growth and development of the OFM, thus leading to the immune function of *PGRP* be restrained. We will further verify this in the following experiments.

Conclusions. As a major pattern recognition receptor, PGRP plays an important role in the innate immune regulation of OFM. In this study, for the first time we obtained and verified the full sequence of the short types of PGRPs gene named it GmPGRP-SC. Its GenBank accession number is MW773839. The transcriptional expression of GmPGRP-SC gene was analyzed in different developmental stages and different tissues of OFM. Results showed some difference with other reported PGRPs from other insects. Perhaps it relates with immunity functions either at specific times or at specific locations. It needs to be further studied, and we will use RNAi method to verify this in the future. Meanwhile we have identified the immune response reduced by B. bassiana, for the first time it was studied the interaction between this fungus and OFM on molecular aspect. All these results have provided a good support for better understand the function of GmPGRP-SC gene in OFM, and also made a foundation for finding target genes and further prevention and control by molecular biology method. This can be applied by interfering the expression of GmPGRP-SC gene. The immune ability of the body to resist fungus can be reduced, so to achieve an effective prevention and control role. It is difficult to control the OFM, therefore, it is one of the effective means to control the occurrence of it and increase the economic benefits of orchard by strengthening the research on the molecular mechanism and regulation mechanism of the innate immunity of OFM.

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Молекулярна ідентифікація білка пептидоглікану короткого типу, GmPGRP-SC від Grapholitha molesta

Білок розпізнавання пептидогліканів (PGRP) є важливим рецептором розпізнавання паттернів, який міститься як у безхребетних, так і у хребетних. Він відіграє важливу роль в антибактеріальному імунітеті, завдяки своїй помітній здатності виявляти та усувати збудника інфекції. Однак PGRP, в основному ідентифіковано з Drosophila melanogaster ma Bombyx mori, а щодо інших сільськогосподарських комах повідомлень мало про їхні функції та механізм. У цьому дослідженні короткотиповий ген PGRP під назвою GmPGRP-SC був ідентифікований у Grapholitha molesta – східної плодожерки (OFM) – на основі аналізу бази даних груп транскрипції OFM з нашої лабораторії та Національного центру інформації з біотехнологій (https://www.ncbi.nlm.nih.gov/). GmPGRP-SC містить домен PGRP і має найтісніший генетичний зв'язок з геном PGRP Leguminivora glycinivorella, відповідно до послідовності та філогенетичного аналізу. Метод ПЛР у реальному часі було використано для аналізу картини його експресії на стадіях розвитку OFM та в різних тканинах личинки OFM. Наразі, відносно рівні експресії гена GmPGRP-SC в OFM були проаналізовані після інфікування Beauveria bassiana. Результати показали, що загальна кДНК від GmPGRP-SC становила 3221 bp (базових, основних пар), а кодуючі області — 2268 bp, що кодують 756 амінокислотних залишків. Рівень експресії GmPGRP-SC був найвищим у стадії лялечки OFM. Водночас, у різних тканинах ОFM його відносна експресія була вищою в епітелії та гемоциті, тоді як іншим стадіям та тканинам притаманна порівняно нижча та з невеликою різницею. Рівень експресії GmPGRP-SC був істотно різним, коли суспензія спор В. bassiana становить 105 конідій/мкл, інфікованих через 48 годин. Тоді, коли суспензія спор В. bassiana становить 107 конідій/мкл, рівень експресії GmPGRP-SC також був різним. Усі ці результати закладуть основу для вивчення ролі та функцій GmPGRP-SC у вродженому імунітеті OFM, а також сприятимуть подальшому вивченню молекулярної взаємодії між ОFM та В. bassiana. Результати досліджень можуть допомогти знайти потенційні молекули-мішені та забезпечити наукову основу для розробки нових біогенних пестицидів та реалізації Зеленої боротьби зі шкідниками (GPM).

Ключові слова: східна плодожерка, вроджений імунітет, Beauveria bassiana, біологічна боротьба зі шкідниками.