UDC 632.78.04/.08

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

Cao Zhishan
PhD Student
Sumy National Agronomy University, Sumy, Ukraine;
School of Resources and Environment, Henan Institute of Science and Technology, Xinxiang, China
ORCID: 0000-0003-3127-4592
caozhishan123@163.com

Cao Jinjun
Doctor (Agricultural Sciences), Associate Professor
School of Resources and Environment, Henan Institute of Science and Technology, Xinxiang, China
ORCID: 0000-0001-5649-0465
cjj1986108@163.com

Zhu Hongxia
PhD Student
Sumy National Agronomy University, Sumy, Ukraine
School of Resources and Environment, Henan Institute of Science and Technology, Xinxiang, China
ORCID: 0000-0003-0113-779X
zhxhg105@163.com

Vlasenko Volodymyr
Doctor (Agricultural Sciences), Professor
Sumy National Agronomy University, Sumy, Ukraine
ORCID: 0000-0002-5535-6747
vlasenkova@ukr.net

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 10^5 conidia/μL infected after 48 h. And when the spore suspension of B. bassiana was 10^7 conidia/μL, 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.*

DOI https://doi.org/10.32845/agrobio.2021.3.7

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). Nowadays 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 drug-resistant 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 ºC, 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
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/ (Ivica & 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 Neighbor-joining 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-1α* 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: pre-denaturation at 95 ℃ for 30s, 95 ºC for 10s, 60 ºC for 30 s, a total of 40 cycles, 95 ºC for 15 s, 60 ºC for 60 s, 95 ºC 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 ºC, 95 % 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 ddH_2O 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 ddH_2O 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,

– ΔCt 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-1α* 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± 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 pI (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

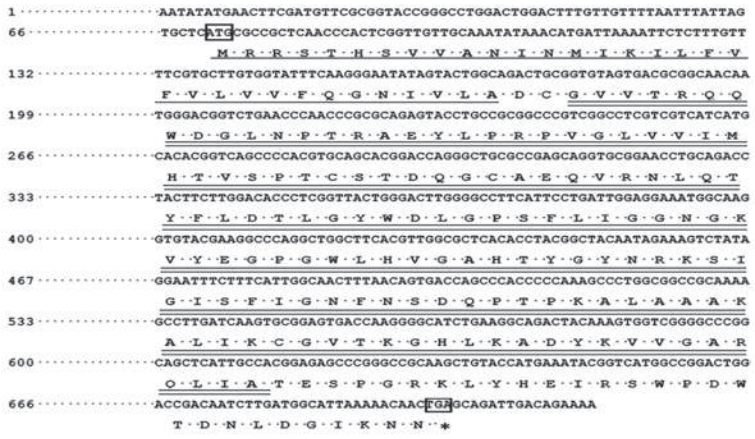

Fig 1. The cDNA and encoding amino acid sequence of the GmPGRP-SC. (The underlined signal indicates the signal peptide sequence; the double underline indicates the PGRP domain, and the start and stop codon are indicated in the boxes)

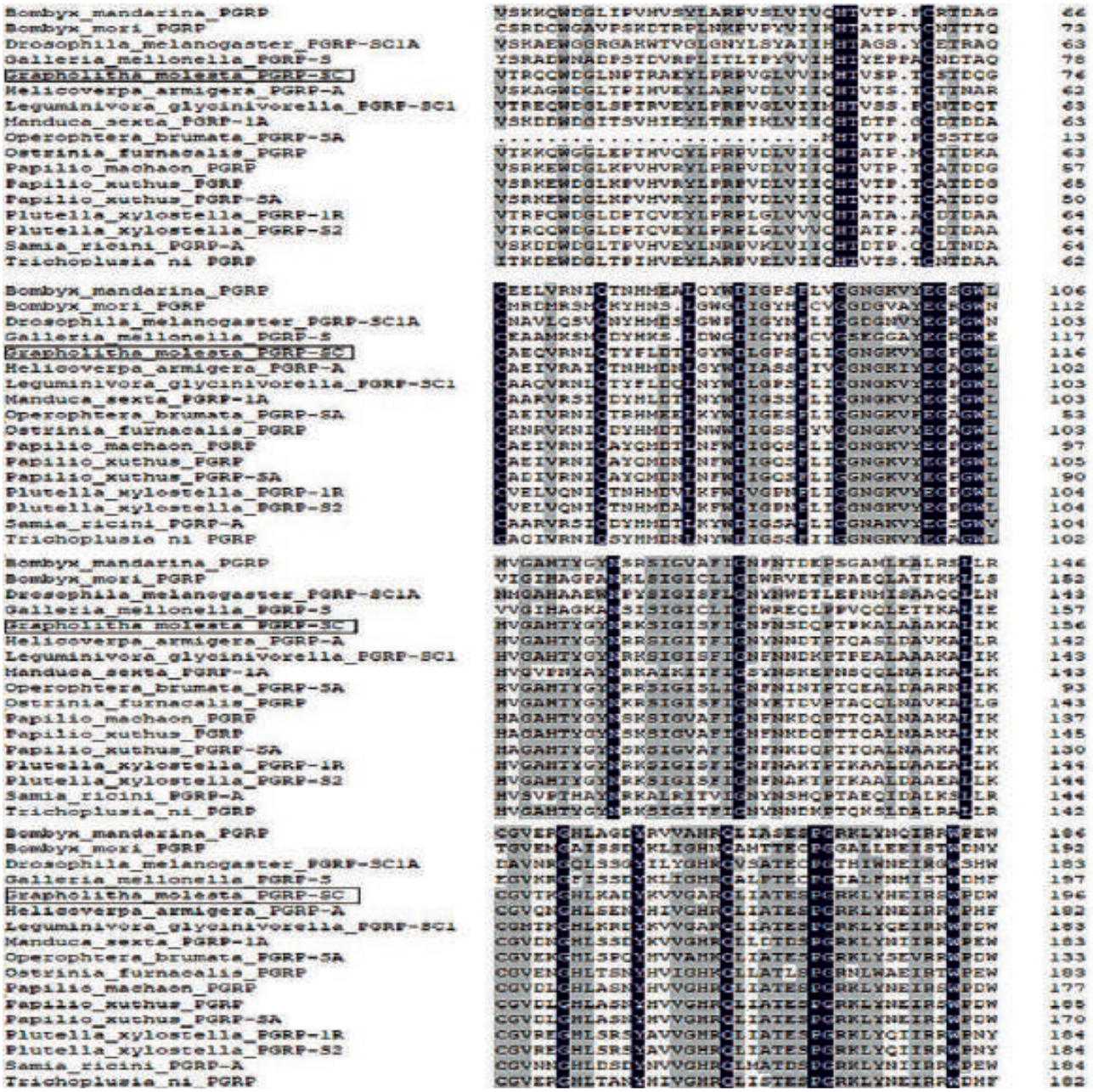

Fig 2. Multiple sequence alignment of *GmPGRP-SC* with the homologs of other insects based on amino acid sequence (The PGRP and correspond GenBank accession numbers are as follows. *Bombyx mandarina* PGRP: XP_028043866.1; *Bombyx mori* PGRP: NP_001036836.1; *Drosophila melanogaster* PGRP-SC1A: CAD89163.1; *Glleria 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-1α* 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

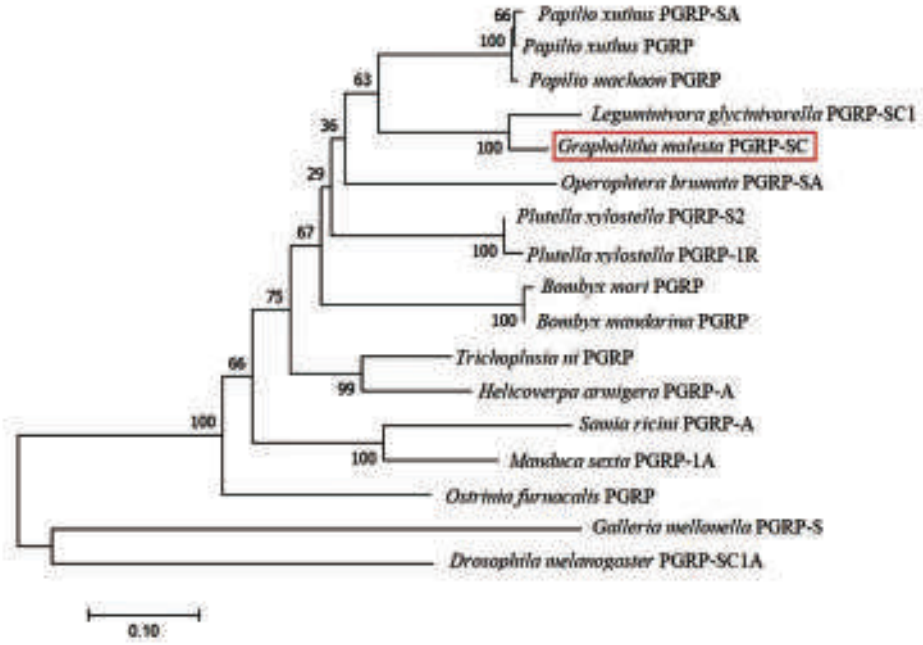

Fig. 3. Phylogenic tree based on amino acid sequence of the PGRP from *Grapholitha molesta* and other insects (Scale bar indicates the genetic distance, the numbers on the branches means confidence coefficient)

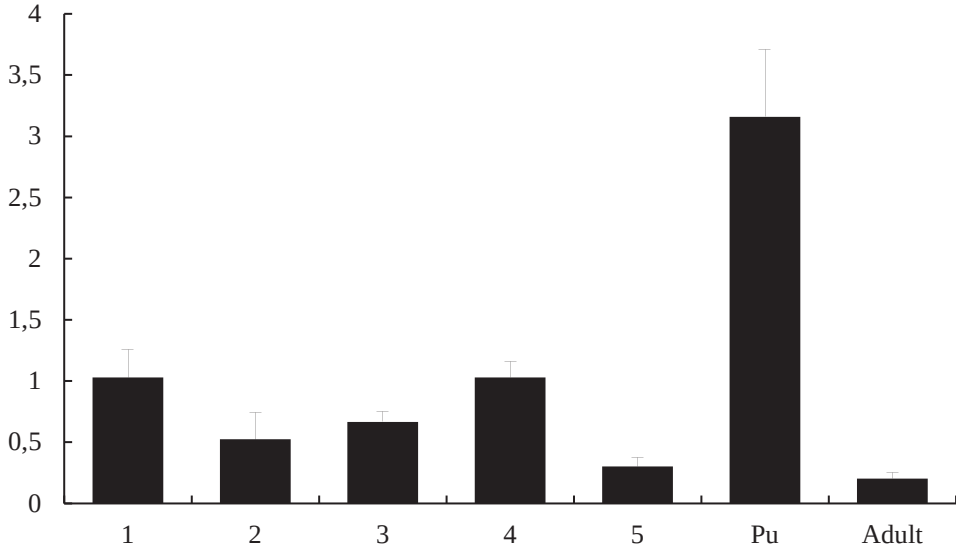

Fig. 4. The temporal expression level of *GmPGRP-SC* gene (Data in the figure are mean ± SD)

Table 2

The temporal expression level of *GmPGRP-SC* gene

Samples	GmPGRP-SC gene Ct	*Ef-1α* gene Ct	Relative expression level	Average	Standard deviation
first instar larvae	22.466	24.091	1.236111073	1.029029912	0.230511995
	22.578	24.129	1.143529957		
	23.271	24.118	0.707448706		
second instar larvae	21.192	22.732	0.77343976	0.525177734	0.218540028
	22.005	22.078	0.440215823		
	22.288	21.679	0.361877618		
third instar larvae	20.093	20.260	0.592918174	0.666356378	0.085862331
	19.971	20.856	0.645391319		
	19.734	20.926	0.760759642		
fourth instar larvae	21.364	22.583	0.94619112	1.028119914	0.135108167
	21.352	22.247	0.954106178		
	21.040	23.046	1.184062445		
fifth instar larvae	20.979	19.596	0.252950515	0.303104737	0.072546125
	20.884	20.150	0.27007543		
	20.368	21.266	0.386288266		
pupal	18.924	23.311	4.402813784	4.27639272	0.54896635
	18.976	21.806	4.246502504		
	19.158	22.093	3.742982119		
adult	18.787	20.788	4.839693538	0.203418039	0.053216785
	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	*Ef-1α* gene Ct	Relative expression level	Average	Standard deviation
head	18.80292702	19.15328789	1.236111073	0.965881618	0.029836818
	18.88503456	19.19662666	1.143529957		
	18.87242699	19.35869598	0.707448706		
epidermis	19.99529457	23.17064857	0.77343976	10.6278559	3.975745188
	18.85653114	23.14433479	0.440215823		
	19.30094528	23.14778519	0.361877618		
fat body	29.18466949	21.02428055	0.592918174	0.003568288	0.000828351
	28.85754013	20.95024109	0.645391319		
	28.51244545	21.41017342	0.760759642		
midgut	18.54099274	18.56934738	0.94619112	0.711292984	0.062598954
	18.76748085	18.47967911	0.954106178		
	18.54175377	18.61497879	1.184062445		
malpighian tubule	25.99302483	22.90022278	0.252950515	0.090309401	0.004558024
	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).

Different tissues from the larvae of the fifth instar at the second day were taken, and *Ef-1α* 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 *GmPGRP-SC* gene in other tissues were not the same. (Tab. 3, Fig. 5).

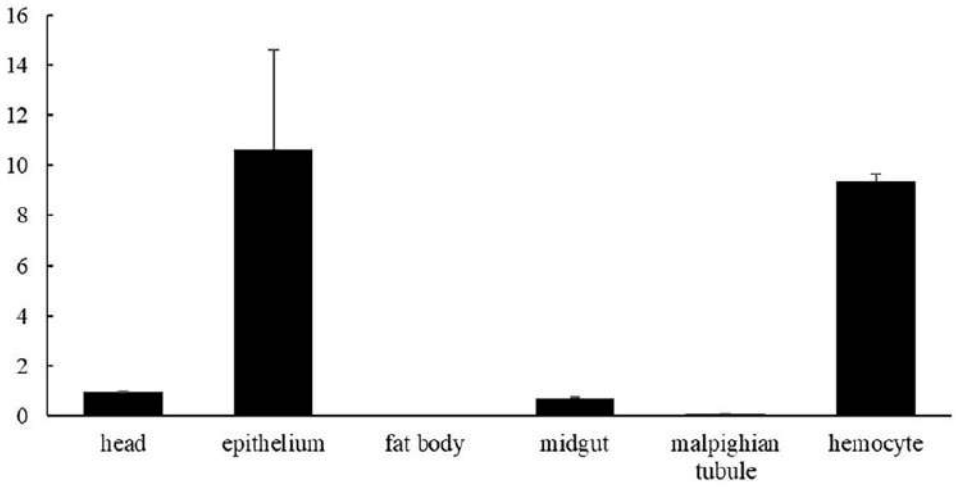

Fig. 5. The relative expression level of *GmPGRP-SC* gene in different tissues of *Grapholitha molesta* larva (Data in the figure are mean ± SD)

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 10^5 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).

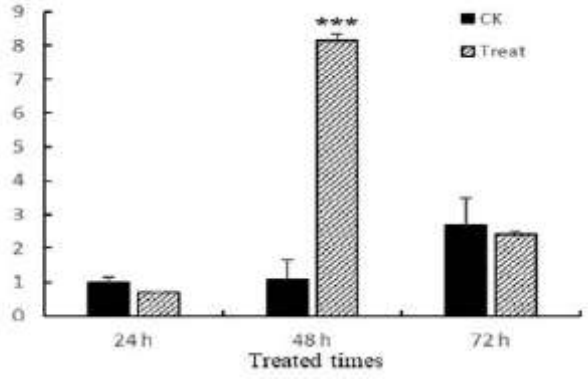

Fig. 6. The temporal expression level of *GmPGRP-SC* gene of different time treated with 10^5 conidia/μL spore suspension of *B. bassiana* (Data in the figure are mean ± SD. The asterisks above bars indicate significance between treatment and the CK determined by the student's t-test, respectively. The 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).

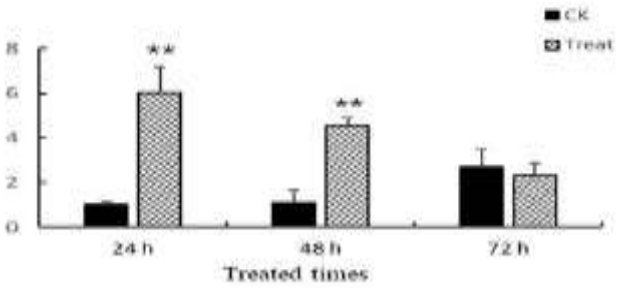

Fig. 7. The temporal expression level of *GmPGRP-SC* gene of different treated with 10^7 conidia/μL spore suspension of *B. bassiana* (Data in the figure are mean ± SD)

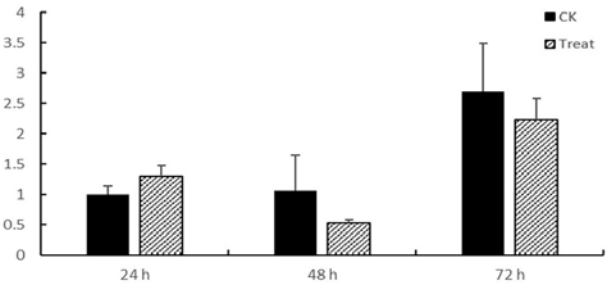

Fig. 8. The temporal expression level of *GmPGRP-SC* gene of different time treated with 10^9 conidia/μL spore suspension of *B. bassiana* (Data in the figure are mean ± SD)

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,

Table 4

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	*Ef-1α* gene Ct	Relative expression level	average	Standard deviation	Student's t test (P) CK and treatment
Ck-24h	21.89202309	21.97925758	1	1	0.140738294	
	21.53461075	22.09150314	1.281125969			
	21.71774483	21.95654106	1.128399756			
Treatment-24h	23.12648392	22.68375015	0.693500568	0.680421291	0.026335	0.005268216
	23.21970367	22.86681175	0.650107053			
	23.11786461	22.59602928	0.697656253			
Ck-48h	22.63484001	20.65943527	1.060431412	1.439278827	0.591494525	
	21.63484001	21.57265854	2.120862824			
	22.53484001	22.10047913	1.136542246			
Treatment-48h	19.61118698	22.82375336	8.340906511	8.155285513	0.194446749	4.83226E-05
	19.64072418	23.00129509	8.171874155			
	19.67987823	22.54035759	7.953075873			
Ck-72h	23.95530319	24.68696785	1.799966838	2.693724964	2.693724964	
	23.07602501	25.15373421	3.31095754			
	23.2326889	25.24067116	2.970250515			
Treatment-72h	20.73429298	22.16456795	2.318965356	2.40994224	0.084880236	0.570824228
	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	*Ef-1α* gene Ct	Relative expression level	average	Standard deviation	Student's t test (P) CK and treatment
Ck-24h	21.89202309	21.97925758	1	1	0.140738294	
	21.53461075	22.09150314	1.281125969			
	21.71774483	21.95654106	1.128399756			
Treatment-24h	22.28517914	22.68375015	6.762721597	6.012059955	1.168196308	0.001996064
	22.31871605	22.86681175	6.607328401			
	22.82055664	22.59602928	4.666129867			
Ck-48h	22.63484001	20.65943527	1.060431412	1.439278827	0.591494525	
	21.63484001	21.57265854	2.120862824			
	22.53484001	22.10047913	1.136542246			
Treatment-48h	20.07825851	22.82375336	6.762721597	4.580062879	0.300287135	0.001204667
	19.88920212	23.00129509	6.607328401			
	19.98667145	22.54035759	4.666129867			
Ck-72h	23.95530319	24.68696785	1.799966838	2.693724964	2.693724964	
	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

Table 6

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

Samples	GmPGRP-SC gene Ct	*Ef-1α* gene Ct	Relative expression level	average	Standard deviation	Student's t test (P) CK and treatment
Ck-24h	21.89202309	21.97925758	1	1	0.140738294	
	21.53461075	22.09150314	1.281125969			
	21.71774483	21.95654106	1.128399756			
Treatment-24h	21.59008408	22.68375015	1.311036896	1.301804097	0.167356775	0.260578719
	21.43052864	22.86681175	1.464353353			
	21.80444145	22.59602928	1.13002204			
Ck-48h	22.63484001	20.65943527	1.060431412	1.439278827	0.591494525	
	21.63484001	21.57265854	2.120862824			
	22.53484001	22.10047913	1.136542246			
Treatment-48h	23.84972191	22.82375336	0.465275442	0.521271989	0.065653954	0.055704075
	23.49849129	23.00129509	0.593527824			
	23.73148727	22.54035759	0.505012702			
Ck-72h	23.95530319	24.68696785	1.799966838	2.693724964	2.693724964	
	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 immune-related 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 *PGRP*s 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.

Acknowledgments

We are very apricate to Professor Zhang Huaijiang for gifting the insects. The research was supported by the Key Scientific and Technological Research Project of Henan Province (N0. 202102110220), Henan engineering research center of biological pesticide and fertilizer development and synergistic application and Aid program for Science and Technology Innovative Research Team in higher Educational Institutions of Henan Province.

References:

1. Artimo, P., Jonnalagedda, M., Arnold, K., Baratin, D., Csardi, G., de Castro, E., Duvaud, S., Flegel, V., Fortier, A., Gasteiger, E., Grosdidier, A., Hernandez, C., Ioannidis, V., Kuznetsov, D., Liechti, R., Moretti, S., Mostaguir, K., Redaschi, N., Rossier, G., Xenarios, I. & Stockinger, H. (2012). ExPASy: SIB bioinformatics resource portal. Nucleic Acids Research, 40(1), 597–603. doi: 10.1093/nar/gks400

2. Armenteros, J. J. A., Tsirigos, K. D., Sonderby, C. K., Petersen, T. N., Winther, O., Brunak, S., Von Heijne, G. & Nielsen, H. (2019). SignalP 5.0 improves signal peptide predictions using deep neural networks. Nature Biotechnology, 37(4), 420 doi: 10.1038/s41587-019-0036-z

3. Blanco, G.A., Malchiodi, E.L. & De Marzi, M.C. (2008). Cellular clot formation in a sipunculan worm: entrapment of foreign particles, cell death and identification of a PGRP-related protein. Journal of invertebrate pathology, 99(2), 156–165.

4. Benelli, G., Lucchi, A., Thomson, D. & Loriatti, C. (2019). Sex pheromone aerosol devices for mating disruption: challenges for a brighter future. Insects, 10, 308.

5. Beutler, B. (2004). Innate immunity: an overview. Molecular Immunology, 40(12), 845–859. doi: 10.1016/j.molimm.2003.10.005

6. Bustin, S. A., Benes, V., Garson, J.A., Jan, H. Jim, H. Mikael, K. Reinhold, M. Tania, N. Pfaffl, M. W. & Shipley, G. L. (2009). The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem, 55(4), 611–622.

7. Cao, J. J. (2015) The expression and functional study of ecdysone signal transduction pathway genes in *Grapholitha molesta.* [Doctor thesis].

8. Dziarski, R. (2003). Recognition of bacterial peptidoglycan by the innate immune system. Cellular and Molecular Life Sciences, 60, 1793–1804.

9. Dziarski, R. & Gupta, D. (2006) The peptidoglycan recognition proteins (PGRPs). Genome Biology, 7(8), 232. doi: 10.1186/gb-2006-7-8-232

10. Du, S.X., Wu, C., Sue, Y.H. Yang, M. & Nathalie, B. (2011). Effects on mass rearing on the olfactory of an invasive fruit month species, *Grapholita molesta* (Busck). [Master thesis].

11. Du, J., Guo, J.T., Zhang, Y.S. & Wu, J.X. (2009). Effect of Temperature on Development and Reproduction of Grapholitha molesta (Busck) (Lepidoptera: Tortricidae). Acta Agriculturae Boreali-occidentalis Sinica, 18(6), 314–318.

12. Fischer, B. & Siedler, F. (2004). Using the Agilent 2100 bioanalyzer for quality control of protein samples prior to MS-analysis. Agilent Technologies.

13. Gao, Y.F. (2013). Immune Function Analysis of PGRP-SC and Cathepsin L Genes in Musca domestica. [Master thesis].

14. Geer, L.Y., Marchler-Bauer, A., Geer, R.C., Han, L.Y., He, J., He, S.Q., Liu, C.L., Shi, W.Y. & Bryant, S.H. (2010). The NCBI BioSystems database. Nucleic Acids Research, 38, 492–496. doi: 10.1093/nar/gkp858

15. Gerardo, N.M., Altincicek, B. Anselme, C., Atamian, H.S., Barribeau, S.M., Martin de, V., Duncan, E.J., Evans, J., Gabaldón, T., Ghanim, M., Heddi, A., Kaloshian, I., Latorre, A., Moya, A., Nakabachi, A., Perez-Brocal, V., Pignatelli, M., Rahbe, Y., Ramsey, J.S. Spragg, C., Tamames, J., Tamarit, D., Tamborindeguy, C. & Vincent-Monegat, C. (2010). Immunity and other defenses in pea aphids, Acyrthosiphon pisum. Genome Biology, 11(2), R21. doi: 10.1186/gb-2010-11-2-r21

16. Gottar, M., Gobert, V., Michel, T. Belvin, M., Duyk, G., Hoffmann, J.A., Ferrandon, D. & Royet, J. (2002). The Drosophila immune response against Gram-negative bacteria is mediated by a peptidoglycan recognition protein. Nature, 416(6881), 640–643.

17. Hultmark, D. (2003). Drosophila immunity: paths and patterns. Current Opinion in Immunology, 15(1), 12–19. doi: doi: 10.1016/S0952-7915(02)00005-5

18. Ivica, L. & Peer, B. (2017). 20 years of the SMART protein domain annotation resource. Nucleic Acids Research, 46(1), 493–496. doi: 10.1093/nar/gkx922

19. Kang, D., Liu, G., Lundström, A., Gelius, E. & Steiner, H. (1998). A peptidoglycan recognition protein in innate immunity conserved from insects to humans. Proceedings of The National Academy of Sciences of The United States of America, 95(17), 10078–10082. doi: 10.1073/pnas.95.17.10078

20. Kanga, L. H. B., Pree, D. J., van Lier, J. L. & Walker, G. M. (2003). Management of insecticide resistance in oriental fruit moth (Grapholita molesta; Lepidoptera: Tortricidae) populations from Ontario. Pest Management Science, 59, 921–927.

21. Kong, W. N., Wang, Y., Guo, Y. F., Chai, X. H., Li, J. & Ma, R. Y. (2020). Behavioral effects of different attractants on adult male and female oriental fruit moths, Grapholita molesta. Pest Management Science, 76(9), 3225–3235. doi: 10.1002/ps.5878

22. Leone, P., Bischoff, V., Kellenberger, C., Hetru, C., Royetd, J. & Roussel, A. (2008). Crystal structure of Drosophila PGRP-SD suggests binding to DAP-type but not lysine-type peptidoglycan. Molecular Immunology, 45, 2521–2530. doi: 10.1016/j.molimm.2008.01.015

23. Lin, C. Q. & Yao, B. (2012). Recent Advance in Digital PCR. Progress in Chemistry, 24(12), 2415–2423

24. Livak, K. J. & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2 (–Delta Delta C(T)) Method. Methods, 25, 402–408.

25. Liu, Y., Shen, D. X., Zhou, F., Wang, G. R. & An, C. J. (2014). Identification of Immunity-Related Genes in Ostrinia furnacalis against Entomopathogenic Fungi by RNA-Seq Analysis, Plos One, 9(1), e86436. doi: 10.1371/journal.pone.0086436
26. Liu W. (2019). Immune response of intestinal reactive oxygen species and peptidoglycan recognition protein genes in Antheraea pernyi. [Doctor thesis].
27. Lu, Y. Z., Su, F. H., Li, Q.L., Zhang, J., Li, Y. J., Tang, T., Hu, Q. H. & Yu, X. Q. (2020). Pattern recognition receptors in Drosophila immune responses. Developmental and Comparative Immunology, 102, 9. doi: 10.1016/j.dci.2019.103468
28. Myllymaki, H., Valanne, S. & Ramet, M., (2014). The Drosophila imd signaling pathway. The Journal of Immunology, 192, 3455–3462
29. Natale, D., Mattiacci, L., Hern, A., Pasqualini, E. & Dorn, S. (2003). Response of female Cydia molesta (Lepidoptera: Tortricidae) to plant derived volatiles. Bulletin of Entomological Research, 93, 335–342. doi: 10.1079/BER2003250
30. Nolan, T., Hands, R.E. & Bustin, S.A. (2006). Quantification of mRNA using real-time RT-PCR. Nature Protocol, 1, 1559–1582.
31. Ochiai, M. & Ashida, M. (1999). A pattern recognition protein for peptidoglycan. Cloning the cDNA and the gene of the silkworm, Bombyx mori. Journal of Biological Chemistry, 274(17), 11854–11858. doi: https://doi.org/10.1074/jbc.274.17.11854
32. Pavsic, J., Zel, J. & Milavec, M (2016). Assessment of the real-time PCR and different digital PCR platforms for DNA quantification. Analytical and Bioanalytical Chemistry, 408(1), 107–121.
33. Pattyn, F., Speleman, F., De Paepe, A. & Vandesompele, J. (2003). RTPrimerDB: the real-time PCR primer and probe database. Nucleic Acids Research, 31(1), 122–123. doi: 10.1093/nar/gkg011
34. Rufer, A. (2010) Therapeutic protein analysis with the Agilent 2100 Bioanalyzer. Biotechniques, 49(3), 669–671.
35. Saranraj, P. & Jayaprakash, A. (2017). A grobeneficial entomopathogenic fungi–Beauveria bassiana: A Review. Asian Journal of Multidisciplinary Research, 3(2), 1051–1087. doi: 10.22192/iajmr.2017.3.2.4
36. Sarker, S., Woo, Y.H. & Lim, U.T. (2020). Laboratory Evaluation of Beauveria bassiana ARP14 Against Grapholita molesta (Lepidoptera: Tortricidae), Current Microbiology, 77(9), 2365–2373. doi: 10.1007/s00284-020-02012-4
37. Soohyun, L., Hwa, S.C., Byungho, L., Yang, J.O., Jeongsu, O., Minjin, K., Sooncheol, L., Byungwook, L., Kang, C. & Sanghyuk, L. (2011). Accurate quantification of transcriptome from RNA-Seq data by effective length normalization. Nucleic Acids Research, 39(2), e9. doi: 10.1093/nar/gkq1015
38. Sudhir, K., Glen, S. & Koichiro, T. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology & Evolution, 33(7), 54. doi: 10.1093/molbev/msw054
39. Sumathipala, N. & Jiang, H.B. (2010). Involvement of Manduca sexta peptidoglycan recognition protein-1 in the recognition of bacteria and activation of prophenoloxidase system. Insect Biochemistry and Molecular Biology, 40, 487–495. doi: 10.1016/j.ibmb.2010.04.008
40. Tanaka, H., Ishibashi, J. Fujita, K., Nakajima, Y., Sagisaka, A., Tomimoto, K., Suzuki, N., Yoshiyama, M., Kaneko, Iwasaki, T., Sunagawa, T., Yamaji, K., Asaoka, A., Mita, K. & Yamakawa, M. (2008). A genome-wide analysis of genes and gene families involved in innate immunity of Bombyx mori. Insect Biochemistry and Molecular Biology, 38, 1087–1110.
41. Telles, G. P., Araujo, G. S., Walter, M. E. M. T., Brigido, M. M. & Almeida, N. F. (2018). Live neighbor-joining. BMC Bioinformatics, 19, 172. doi: 10.1186/s12859-018-2162-x
42. Tzou, P., De Gregorio, E. & Lemaitre, B. (2002). How Drosophila combats microbial infection: a model to study innate immunity and host-pathogen interactions. Current Opinion in Microbiology, 5, 102–110.
43. Vandesompele, J.D.P.K., Pattyn. F., Poppe B, Roy, N.V., Paepe□A.D. & Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology, 3, 7. doi: 10.1186/gb-2002-3-7-research0034
44. Wang, L. & Wang, Q. (2011). Application of SPSS in Teaching of Variance Analysis. 2nd International Conference on Education and Sports Education, 1.
45. Werner, T., Liu, G., Kang, D.W., Ekengren, S., Steiner, H. & Hultmark, D. (2000). A family of peptidoglycan recognition proteins in the fruit fly Drosophila melanogaster. Proceedings of The National Academy of Sciences of The United States of America, 97(25), 13772–13777
46. Wiegmann, B.M., Trautwein, M.D., Winkler, I.S., Barr, N.B., Kim, J.W., Lambkin, C. Bertone, M.A., Cassel, B.K., Bayless, K.M. & Heimberg, A.M. (2011). Episodic radiations in the fly tree of life. Proceedings of the National Academy of Sciences of the United States of America, 108, 5690–5695.
47. Yang, P.J., Zhan, M.Y., Ye, C., Yu, X.Q. & Rao, X.J. (2017). Molecular cloning and characterization of a short peptidoglycan recognition protein from silkworm Bombyx mori. Insect Molecular Biology, 1, 12–33. doi: 10.1111/imb.12330.
48. Zaidman-Remy, A., Poidevin. M., Herve, M., Welchman, D.P., Paredes, J.C., Fahlander, C., Steiner, H., Mengin-Lecreulx, D. & Lemaitre, B. (2011). Drosophila immunity: analysis of PGRP-SB1 expression, enzymatic activity and function. PLoS. ONE, 6, e17231. doi: 10.1371/journal.pone.0017231
49. Zhao, P., Xia, F., Jiang, L., Guo, H. Z., Xu, G. W., Sun, Q., Wang, B. B., Wang, Y. M., Lu, Z. Y. & Xia, Q. Y. (2018). Enhanced antiviral immunity against Bombyx mori cytoplasmic polyhedrosis virus via overexpression of peptidoglycan recognition protein S2 in transgenic silkworms. Developmental & Comparative Immunology, 87, 84–89.
50. Zhang, F.M., Shan, S.J., Xu, X.Y., Wang, Y., Zhang, Y.H. Yin, M. & Yang, G.W. (2019). Molecular characterization and expression analysis of two peptidoglycan recognition proteins (CcPGRP5, CcPGRP6) in larvae ontogeny of common carp Cyprinus carpio L. and upon immune stimulation by bacteria. BMC veterinary Research, 15, 10. doi: 10.1186/s12917-018-1744-1.
51. Zhang, X.Y. Chen, D., Zhang, S.Y., Wei, D. & Wang, J.J. (2020). Cloning and functional characterization of the peptidoglycan recognition protein gene BdPGRP-SB1 in Bactrocera dorsalis (Diptera: Tephritidae). Acta Entomologic Sinica, 63(9), 1070–1080. doi: 10.16380/j.kcxb.2020.09.004

***Цао Жішан**, аспірантка, Сумський національний аграрний університет, м. Суми, Україна; Школа ресурсів і довкілля, Хенанський інститут науки і технологій, м. Ксінсянг, КНР*

***Цао Цзиньцзюнь**, доктор сільськогосподарських наук, доцент, Школа ресурсів і довкілля, Хенанський інститут науки і технологій, м. Ксінсянг, КНР*

***Чжу Хонгсія**, аспірантка, Сумський національний аграрний університет, м. Суми, Україна; Школа ресурсів і довкілля, Хенанський інститут науки і технологій, м. Ксінсянг, КНР*

***Власенко Володимир Анатолійович**, доктор сільськогосподарських наук, професор, Сумський національний аграрний університет, м. Суми, Україна*

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 та 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 OFM 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 B. bassiana становить 105 конідій/мкл, інфікованих через 48 годин. Тоді, коли суспензія спор B. 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 OFM та B. 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.*