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The *PLA2* gene mediates the humoral immune responses in *Bactrocera dorsalis* (Hendel)

Qiujia Li, Xiaolong Dong, Weiwei Zheng, Hongyu Zhang*

Key Laboratory of Horticultural Plant Biology (MOE), State Key Laboratory of Agricultural Microbiology, Institute of Urban and Horticultural Entomology, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, China

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ABSTRACT

The phospholipase A2 (*PLA2*) gene encodes the enzyme that catalyzes the hydrolysis of phospholipids (PLs) from the sn-2 position. However, little is known about its role in humoral immune responses. In this study, we investigated the expression profile of *PLA2* in different tissues and developmental stages in *Bactrocera dorsalis* (Hendel), and the results showed that the transcriptional level of *PLA2* was high in the egg and mature stage and in the testis tissue. Bacterial infection increased the expression of *PLA2*, and the highest degree of up-regulation appeared in the fat body. Silencing *PLA2* influenced the expression of immune-related genes, including *MyD88* and *defensin* in the Toll pathway and *relish* and *diptericin* in the Imd pathway. Moreover, the expression of *MyD88* and *defensin* was down-regulated significantly in the ds-*PLA2* group compared with those in the ds-*egfp* group when *B. dorsalis* was infected with *L. monocytogenes* and *S. aureus*, indicating that *PLA2* was involved in the activation of the Toll pathway. Meanwhile, infection with *L. monocytogenes* and *E. coli*, which activate the Imd pathway, does not increase the mRNA levels of *relish* and *diptericin* in the ds-*PLA2* group as severely as it increases those in the ds-*egfp* group, indicating that the Imd pathway was also repressed after silencing *PLA2*. Notably, the development of lipid droplets in fat body cells was influenced by silencing *PLA2*, implying that *PLA2* affects the function of fat body tissue. These results suggest that the *PLA2* gene may mediate humoral immune responses by reducing lipid storage in fat body cells in *B. dorsalis*.

1. Introduction

To defend against various microorganisms, such as bacteria, fungi or viruses, insects have developed a powerful and diversified innate immunity system, which comprises both cellular and humoral immune responses (Ratcliffe et al., 1985). Cellular immunity refers to hemocyte-mediated immune responses, including phagocytosis, nodulation, and encapsulation (Gillespieand et al., 2003). Humoral immune responses are characterized by the release of various antimicrobial peptides which are mainly produced by fat body cells (Lavine and Strand, 2002). Studies in *Drosophila melanogaster* have revealed that production of antimicrobial peptides results largely from the activation of two distinct pathways: the Toll pathway and the immune deficiency (Imd) pathway (Hultmark, 2003).

Different groups of microbes are recognized by different pattern recognition receptors that activate specific signaling pathways and induce the appropriate immune reaction to these infections (Kurata, 2004). Gram-positive bacteria can be divided into two types based on their peptidoglycans. Lysine-type peptidoglycan bacteria are strong inducers of the Toll pathway, while DAP-type peptidoglycan Gram-positive bacteria, including the *Bacillus* and *Listeria* genera, activate both the Toll and Imd pathways (Neyen et al., 2014). The Imd pathway is mainly responsible for detecting and responding to Gram-negative bacteria.

Phospholipase A2 (PLA2) is responsible for releasing the fatty acid moiety from the sn-2 position of phospholipids (Dennis et al., 2011). PLA2 acts in a wide array of biological processes, such as lipid digestion, cellular membrane remodeling, signal transduction, host immune defense and production of various lipid mediators (Valentin and Lambeau, 2000). Many papers have demonstrated the important role of *PLA2* in insect cellular immune responses. The activity of the PLA2 enzyme increased after bacterial challenge in *Manduca sexta* (Tunaz et al., 2003). The insect pathogenic bacteria in the genera *Photorhabdus* and *Xenorhabdus* exert immunodepressive actions in part by inhibiting PLA2 (Kim et al., 2005; Park

* Corresponding author.
E-mail address: hongyu.zhang@mail.hzau.edu.cn (H. Zhang).

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and Aliza, 2005). Inhibiting PLA2 activity reduced nodulation reactions to bacterial infections in *M. sexta* (Miller et al., 1994), *Tribolium castaneum* (Shrestha et al., 2009) and *Spodoptera exigua* (Park et al., 2015). However, little is known about the function of *PLA2* in the humoral immune response in insects.

The oriental fruit fly *Bactrocera dorsalis* (Hendel), which is one of the most destructive pests found throughout South East Asia and in multiple Pacific Islands, causes severe economic loss to over 250 fruit crops (Chen et al., 2015; Li et al., 2011). In this study, the expression profiles of the *PLA2* gene in different developmental stages and tissues were detected by qRT-PCR. RNAi was employed to demonstrate the effects of *PLA2* on the activation of Toll and Imd pathways as well as the phenotypic characters of fat body cells. These results can broaden our knowledge of innate immunity in insects.

2. Material and methods

2.1. Insects

The adults of *B. dorsalis* were reared as described by Li et al. (2011), as follows: newly emerged flies were fed with artificial diets consisting of 2.5% yeast extract, 7.5% sugar, 2.5% honey, 0.5% agar and 87% water at 28 °C, and a photoperiod of 12 h light: 12 h dark. Eggs and the hatched larvae were supplied with bananas.

2.2. Expression profile analysis

Insects at different developmental stages were collected and washed using DEPC water, including eggs, the first, second, and third instar larvae, pupae. New emerged flies were collected 5 h after emergence. Mature flies were collected 20 days after emergence. Each sample contained 20 individuals.

For tissue sample collection, mature adults were dissected under a stereomicroscope (Olympus SZX12, Tokyo, Japan) in DEPC water. Tissues (head, midgut, hingut, fat body, testis and ovary) of 30 flies were collected for total RNA isolation. The experiments were performed in triplicate. *PLA2* expressions at different stages and different tissues were examined using qRT-PCR.

2.3. Bacterial infection

Three microbes were used to test the response of *PLA2* to microbial pathogen infection in *B. dorsalis*. *Listeria monocytogenes* is a gram-positive bacterium possessing a DAP-type peptidoglycan that can activate both the Toll and Imd pathways (Ayres et al., 2008). *Staphylococcus aureus* is a Lysin-type gram-positive bacterium and a strong inducer of the Toll pathway. *Escherichia coli*, which is gram-negative, has previously been applied in research of the Imd pathway in *Drosophila* (Neyen et al., 2014). Importantly, the infecting temperatures of these three bacteria were similar to the fly rearing temperature of 28 °C.

L. monocytogenes was grown for 15 h in BHI medium at 29 °C without shaking. *S. aureus* and *E. coli* were cultured overnight in LB-medium at 37 °C with shaking at 220 rpm/min. Bacterial pellets were collected by centrifuging and diluted in PBS (Gendrin et al., 2013). Bacteria were injected into the hemocoel from the coxal cavity at a dose of 4000 colony-forming units (CFUs) per fly.

Microinjection was performed using an InjectMan NI2 (Eppendorf) combined with a FemtoJet (Eppendorf) microinjection system. The glass capillary for microinjection was made using 50-ml glass micropipettes with a puller at heater level 60.4 (PC-10, Narishige, Tokyo, Japan).

2.4. dsRNA preparation

HT115(DE3) strain and the l4440 plasmid were used to express dsRNAs. The recombinant l4440 plasmids were constructed as described by Li (Li et al., 2011). L4440 plasmids containing *egfp* and *PLA2* fragments were transformed into HT115(DE3) competent cells, respectively. The *PLA2* fragment was obtained from the sequence information from our previous transcriptome of *B. dorsalis* (Zheng et al., 2012) and the primer sequences are shown in Table 1. After transformation, single colonies of HT115(DE3) were cultured in LB at 37 °C with shaking at 220 rpm overnight. The culture was diluted 100-fold in 800 ml of 2 × YT supplemented with 75 mg/ml ampicillin plus 12.5 mg/ml tetracycline and cultured at 37 °C to OD600 = 0.4, 0.4 mM IPTG was added to induce the synthesis of T7 polymerase, and the bacteria were incubated with shaking for an additional 5 h at 37 °C.

Total nucleic acids were extracted as described by Timmons (Timmons et al., 2001). Briefly, cell pellets were collected by centrifugation at 5000 × g for 10 min and resuspended in 1 M ammonium acetate/10 mM EDTA plus the same volume of phenol: chloroform: isoamyl alcohol (25:24:1). The samples were incubated at 65 °C for 30 min. After centrifuging at 12,000 × g for 15 min, the upper phase was mixed with isopropanol and incubated at −20 °C overnight. The nucleic acid pellets were obtained by centrifuging at 12,000 × g for 30 min. After being resuspended in DEPC-treated H_2O, the nucleic acids were treated with RQ1 RNase-free DNase (Promega) and RNase A solution (Promega). The concentrations of dsRNA solutions were determined by NanoDrop 1000 (Thermo). The dsRNAs were also loaded onto a 2% agarose gel, stained with ethidium bromide, and photographed.

2.5. Feeding bioassay

Flies that emerged within 5 days were collected and reared in a 17 cm × 8 cm × 7 cm box. Each treatment contained 50 flies (sex ratio 1:1), which were dehydrated/starved for 24 h before feeding with dsRNA. The artificial diet was placed in glass dishes 3.2 cm in diameter and covered by 800 μl of dsRNA solution (300 ng/μl). The flies were fed the artificial diet supplemented with dsRNA for 6 h and then returned to a normal artificial diet. Each treatment contains three biological replicates. In the feeding experiments, *egfp* dsRNA was used as the control.

Table 1
PCR primers used for RT-PCR and qRT-PCR analyses.

Primer	Sequence (5′ to 3′orientation)	Purpose
Q*16s*-L	CTCGTCCAACCGTTCATACC	Real-time qPCR
Q*16s*-R	CTGACCTGCCCACTGAAGTT	Real-time qPCR
QPLA2-R	ACGACCCACACTGCGTAATG	Real-time qPCR
QPLA2-L	CAGGAGCAGCACCATCGTAT	Real-time qPCR
Q*MyD88*-L	GCAACAATGATGACCCGATGC	Real-time qPCR
Q*MyD88*-R	CAACCGTGCAAATGTCGCTG	Real-time qPCR
Q*relish*-L	TGGAGCGGCGATGAAAACAA	Real-time qPCR
Q*relish*-R	TACGCTTGGAATTGGCACCA	Real-time qPCR
Q*defensin*-L	GCGTTTTCTGCCTGTTCCAA	Real-time qPCR
Q*defensin*-R	GCACATCCAATTCTGCGTCA	Real-time qPCR
Q*diptericin*-L	CCCAAAGACAGCCTCAGTTCA	Real-time qPCR
Q*diptericin*-R	TTGCGAATACTGTCCGGTGG	Real-time qPCR
S*egfp*-L	CGAGCTC ACGTAAACGGCCACAAGTTC	Plasmid construction
S*egfp*-R	CCCAAGCTTAAGTCGTGCTGCTTCATGTG	Plasmid construction
SPLA2-L	CGAGCTCGGTAACTTGCTTGTGGATCA	Plasmid construction
SPLA2-R	CCCAAGCTTCGATTGTTGTGCATATAGGC	Plasmid construction
SPLA2′-L	CGAGCTCCTGTCCCGGTTTCAATAGTT	Plasmid construction
SPLA2′-R	CCCAAGCTTGATCCACAAGCAAGTTACCA	Plasmid construction

Primers starting with an S were used to get PCR fragment for L4440 plasmid construction. Primers starting with a Q were designed for qRT-PCR. The underlined part indicates enzyme restrict site.

2.6. The impact of PLA2 silencing on the Toll/Imd pathways in B. dorsalis

To explore the impact of *PLA2* silencing on the Toll and Imd pathways in *B. dorsalis*, bacteria were injected into individuals after feeding with dsRNAs. Three experimental groups were used: (1) negative control group (CK group), injected with PBS 24 h after feeding ds-*egfp*; (2) positive control group (ds-*egfp* group), injected with bacteria 24 h after feeding ds-*egfp*; (3) treatment group (ds-*PLA2* group), injected with bacteria 24 h after feeding ds-*PLA2*. Samples were collected 24 h after bacterial infection. The mRNA levels of *MyD88* and *defensin* in the Toll pathway were detected after injecting *L. monocytogenes* and *S. aureus* by real-time PCR, while the expression levels of *relish* and *diptericin* in the Imd pathway were tested after *L. monocytogenes* and *E. coli* infection, respectively.

After infection with *L. monocytogenes*, the dead flies were counted every day until the mortality rate was 100%, and then the median time to death (MTD) was calculated. Each treatment contained 30 flies (sex ratio = 1:1).

2.7. Real-time PCR

For each treatment, 10 flies were collected for total RNA extraction. Total RNAs were extracted with RNAiso™ Plus (TaKaRa). First-strand cDNA was produced using a PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa). For RT-PCR, 1 mg of total RNA was used. Real-time PCR was performed using iTaq™ Universal SYBR Green Supermix (BioRad) on a BioRad iCycler according to the manufacturer's instructions. For the internal control gene, 16S rRNA was chosen (Li et al., 2011). The thermocycler conditions were 95 °C for 30 s, followed by 40 cycles at 95 °C for 15 s and 60 °C for 30 s. The thermocycler conditions for the melting curve analysis were 55 °C for 60 s, followed by 81 cycles starting at 55 °C for 10 s with a 0.5 °C increase each cycle. The relative gene expression data were analyzed using the $2^{-\Delta\Delta CT}$ method as described by (Livak and Schmittgen, 2001). All of the primers for real-time PCR were designed using Primer 3 software (http://bioinfo.ut.ee/primer3-0.4.0/) based on the sequence information from the transcriptome of *B. dorsalis* sequenced by us previously (Zheng et al., 2012). The primer sequences are shown in Table 1.

2.8. Staining and microscopy

Five days after treatment with dsRNA, flies were collected for fat body staining. Fat body staining was performed as described by Tian (Tian et al., 2011) with slight modifications.

For fat body staining, adult flies were dissected in PBS, and the tissues were fixed in 4% formaldehyde for 20 min at room temperature. The tissues were then rinsed twice with PBS and incubated for 30 min at a 1:1000 dilution of 0.5 mg/ml Nile red (Sigma) in PBS. After rinsing twice with PBS, the nuclei were stained with DAPI. Images were captured on an Olympus IX71 microscope driven by cellSens Dimension software (Olympus, Japan). All images were imported into and processed in Adobe Photoshop (Adobe, USA).

2.9. Statistical analysis

Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). All of the data from experimental replicates were analyzed by one-way analysis of variance (ANOVA) and Student's *t*-test. The data was processed as described by Li (Li et al., 2011).

3. Results

3.1. The expression profiles of PLA2 in B. dorsalis

The expression profiles of *PLA2* at different developmental stages were analyzed by qRT-PCR. The *PLA2* mRNA transcripts were detectable at all of the developmental stages tested. The highest expression appeared at the egg and sexually mature adult stages. The transcripts of *PLA2* showed no significant difference among the three larval stages, while the mRNA level at the pupa stage was higher than that at the 1st and 2nd larval stages (Fig. 1A). Meanwhile, the expression of *PLA2* in mature adults showed an increase compared with the newly emerged flies.

The expression profiles of the *PLA2* gene in six different tissues of the adults were also analyzed. Our results showed that the expression levels of *PLA2* in the testis, fat body and head were higher than those in other tissues, with the highest expression in the testis. The midgut and hindgut showed no significant difference in *PLA2* expression (Fig. 1B).

3.2. Responses of the PLA2 gene to microbial pathogen infections in B. dorsalis

The relative expression of the *PLA2* gene was detected following the microbial pathogen infections in *B. dorsalis*. After injection with *E. coli* or *S. aureus*, the expression of *PLA2* increased by 83% and 64% at 24 h postinfection, respectively. In addition, the transcription level of *PLA2* in the *L. monocytogenes*-injected group was 2.92-fold higher than that in the PBS-injected group (Fig. 2A). This result indicated that bacterial challenge significantly increases *PLA2* expression.

The mRNA levels of *PLA2* in different tissues were measured 24 h after injecting microbial pathogens. As the results show, the expression of *PLA2* was up-regulated in all tested tissues. In the three types of bacterial infections, the maximum increase in *PLA2* expression appeared in the fat body (Fig. 2B). After injecting with *E. coli*, the expression of *PLA2* in the fat body increased by 74.4%. The transcription levels of *PLA2* in the *S. aureus*- and *L. monocytogenes*-injected groups were 2.96- and 6.87-fold higher than that in the PBS-injected group, respectively.

3.3. PLA2 silencing impaired the expression of immune-related genes in B. dorsalis

After feeding ds-*PLA2* for 6 h, the relative expression of target genes was detected for 5 consecutive days (Fig. 3A). The results showed that *PLA2* gene transcription was reduced by 44%, 65%, 45% and 16% in the first 4 days, respectively. At day 5, there was no significant difference between two groups in terms of *PLA2* expression.

The mRNA levels of *PLA2* in different tissues were measured 24 h after feeding ds-*PLA2*. According to the results, the relative expression of *PLA2* was down-regulated in all tested tissues. The maximum decrease in target gene expression appeared in the fat body, which showed down-regulation of 61% compared with the control group (Fig. 3B).

The relative expression levels of *MyD88* and *defensin* in the Toll pathway decreased significantly after ds-*PLA2* feeding. The maximum down-regulation of *MyD88* appeared in the 1st day with a drop of 50%, while the *defensin* gene decreased most in the 2nd day, and the maximum down-regulation was 84%. When examining *relish* and *diptericin* in the Imd pathway, both of the genes showed the maximum decrease on the 1st day after *PLA2* silencing, which was 54% and 75%, respectively (Fig. 3C).

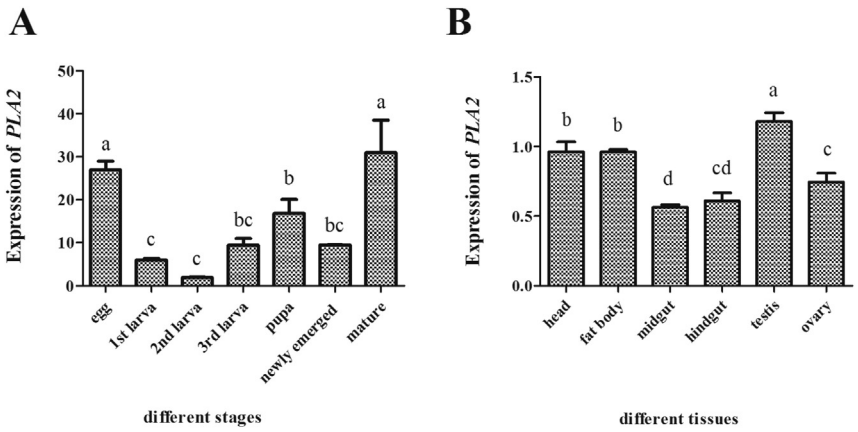

Fig. 1. The expression profiles of *PLA2* in *Bactrocera dorsalis*. **(A)** The expression of *PLA2* at different developmental stages. **(B)** The expression of *PLA2* in different tissues. Different letters indicate a significant difference in the expression at the level of $P < 0.05$.

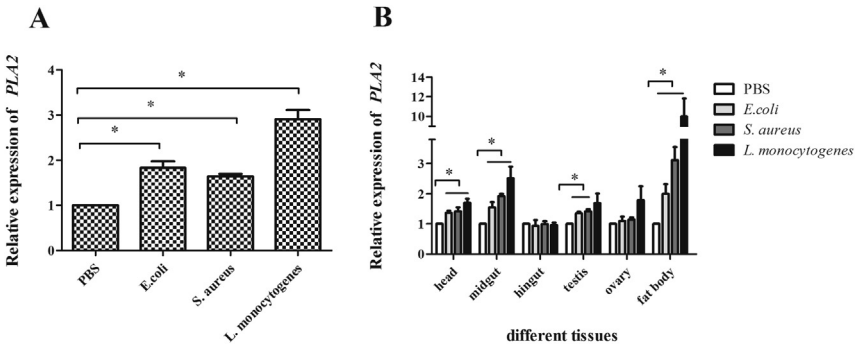

Fig. 2. Responses of the *PLA2* gene to microbial pathogen infections in *B. dorsalis*. **(A)** Relative expression of the *PLA2* gene in *B. dorsalis* adults after microbial pathogen infections. **(B)** Relative expression of the *PLA2* gene in different tissues of *B. dorsalis* after microbial pathogen infections. The normalized target gene expression is reported relative to the expression after injecting with PBS, which was set to 1. Three independent biological replicates were performed. * indicates a statistically significant difference between the treatment group and control group ($P < 0.05$).

To test whether potential off-target effects of ds-*PLA2* exist, we synthesized a new dsRNA for *PLA2* (ds-*PLA2'*) based on another sequence fragment of *PLA2* and tested its influence on the expression of immune-related genes according to methods as described by Seinen et al. (Seinen et al., 2011). The results were showed in Fig. 3D and E. According to the data, similar results were achieved as those were shown in ds-*PLA2* treated group. After feeding with ds-*PLA2′*, the expression of target genes was detected for 5 consecutive days. The results showed that *PLA2′* gene transcription was reduced by 48%, 70%, 37% and 25% in the first 4 days, respectively (Fig. 3D). At day 5, there was no significant difference between two groups in terms of *PLA2′* expression. The relative expression levels of *MyD88* and *defensin* in the Toll pathway, *relish* and *diptericin* in the Imd pathway decreased significantly after ds-*PLA2′* feeding. The maximum down-regulation of *relish* and *MyD88* all appeared in the 1st day with a drop of 63% and 68% (Fig. 3E). When examining the expression level of *defensin* and *diptericin*, both of the genes showed the maximum decrease on the 2nd day after *PLA2′* silencing, which was 95% and 86%, respectively. Those results suggested that there was no off-target effects of ds-*PLA2* to this experiment and *PLA2* silencing impaired the expression of immune-related genes in *B. dorsalis*.

3.4. *PLA2* silencing influences the humoral immune response in *B. dorsalis*

The mRNA level of *MyD88* and *defensin* in the Toll pathway was measured 24 h after injection of *L. monocytogenes* and *S. aureus* following feeding ds-*PLA2*. The expression levels of both genes in the ds-*egfp* group were significantly up-regulated compared with those of the CK group (Fig. 4A). After injecting *L. monocytogenes* and *S. aureus*, the increase in *MyD88* transcription in the ds-*egfp* group was 1.78- and 1.81-fold higher than that in the CK group, while the mRNA level of *defensin* was 60.62 and 16.26 times higher than that in the CK group. However, in the ds-*PLA2* group, in which *PLA2* had been silenced, the mRNA levels of *MyD88* and *defensin* appeared to decrease compared with the levels in the ds-*egfp* group. The mRNA level of *MyD88* appeared to decrease by 47% and 57% after injecting *L. monocytogenes* and *S. aureus*, respectively, and *defensin* showed a reduction of approximately 66% and 58% (Fig. 4A).

Similar changes took place in the Imd pathway. The expression of *relish* and *diptericin* was detected at 24 h after *L. monocytogenes* and *E. coli* infections, respectively. In the ds-*egfp* group, both *relish* and *diptericin* showed significant increases compared with the CK group (Fig. 4B). After injecting *L. monocytogenes* and *E. coli*, transcripts of *relish* showed an up-regulation of 2.41- and 3.08-fold over the CK group, while the expression of *diptericin* showed an increase of 11.30- and 5.18-fold over the CK group. Similar to the target genes in the Toll pathway, the mRNA levels of *relish* and *diptericin* in the ds-*PLA2* group showed decreases compared with the ds-*egfp* group. The down-regulation of *relish* and *diptericin* in the ds-*PLA2* group occurred with 60% and 44% reductions after injecting *L. monocytogenes* compared with the ds-*egfp* group. After injection of *E. coli*, both *relish* and *diptericin* in the ds-*PLA2* group showed reductions of

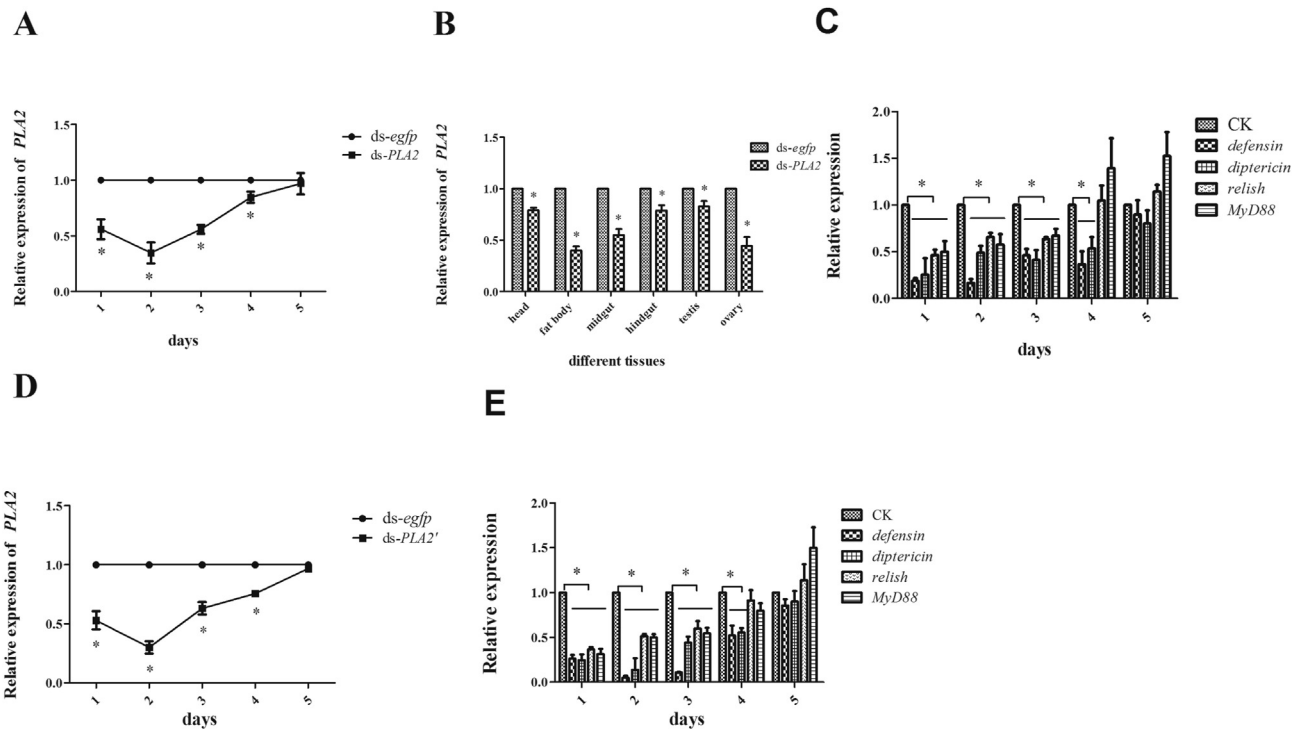

Fig. 3. Influence of *PLA2* silencing to related genes in Toll/Imd pathways. **(A)** The relative expression of *PLA2* after feeding with ds-*PLA2* for 5 days. **(B)**. The relative expression of *PLA2* in different tissues 24 h after feeding ds-*PLA2*. **(C)** The relative expression of *MyD88*, *defensin*, *relish* and *diptericin* within 5 days of feeding flies with ds-*PLA2*. **(D)** The relative expression of *PLA2′* after feeding with ds-*PLA2′* for 5 days. **(E)** The relative expression of *MyD88*, *defensin*, *relish* and *diptericin* within 5 days of feeding flies with ds-*PLA2′*. The normalized target gene expression is reported relative to the expression after feeding with ds-*egfp*, which was set to 1. Three independent biological replicates were performed. * indicates a statistically significant difference between the treatment group and the control group ($P < 0.05$).

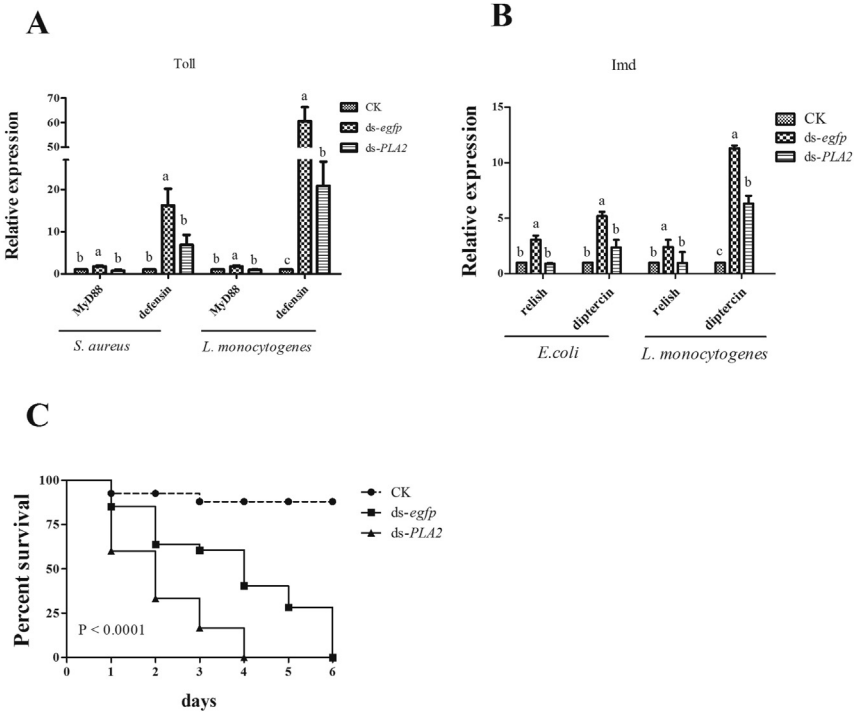

Fig. 4. *PLA2* silencing affected the activation of the humoral immune response. **(A)** The relative expression of *MyD88* and *defensin* 24 h after *L. monocytogenes* and *S. aureus* infections. **(B)** The relative expression of *relish* and *diptericin* 24 h after injecting with *L. monocytogenes* and *E. coli*. **(C)** The survival rate of *B. dorsalis* postinfection with *L. monocytogenes* after feeding with ds-*PLA2*. The normalized target gene expression is reported relative to the expression of the CK group, which was set to 1. Three independent biological replicates were performed for each treatment. Different letters indicate a significant difference in the expression of each target gene among three groups ($P < 0.05$).

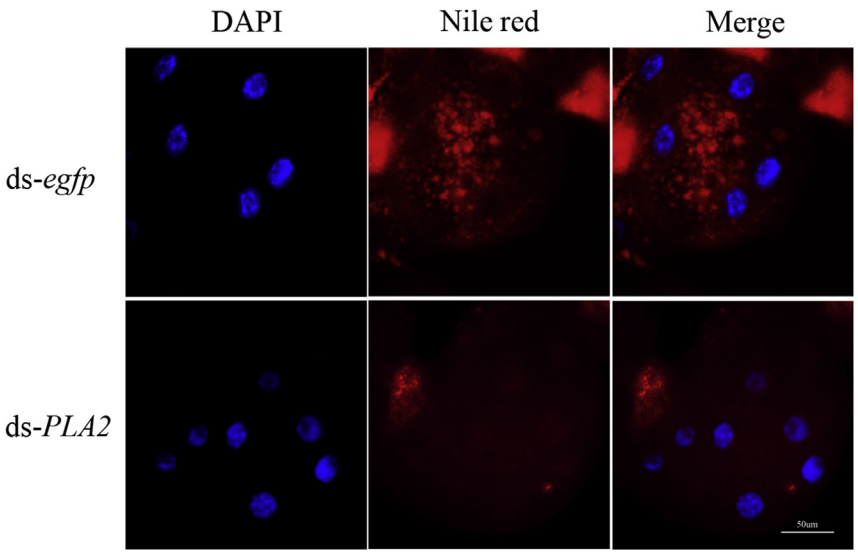

Fig. 5. *PLA2* silencing led to phenotypic changes in fat body cells. Lipid droplets labeled with Nile red (red) in fat body cells from the ds-*PLA2* group and ds-*egfp* group. Nuclei were stained with DAPI (blue). Images were captured on an Olympus IX71 microscope driven by cellSens Dimension software (Olympus, Japan). All images were imported into and processed in Adobe Photoshop (Adobe, USA).

approximately 71% and 54% compared with the ds-*egfp* group (Fig. 4B).

3.5. *PLA2* silencing accelerated the death of *B. dorsalis* caused by pathogen *L. monocytogenes* infection

After injecting with *L. monocytogenes*, the median time to death (MTD) of each group was also determined. As the results show, *PLA2* silencing increased the death rate of *B. dorsalis* after infection with *L. monocytogenes*. The MTD caused by *L. monocytogenes* infection was approximately 4 days in the ds-*egfp* group, while the MTD was approximately 2 days in the ds-*PLA2* group (Fig. 4C).

3.6. *PLA2* silencing reduced lipid storage in fat body cells

The morphology of the fat body was also examined by Nile red staining. The fat body is the adipose tissue of insects and also has liver-like activity (Hoffmann et al., 1993). Lipid storage was significantly reduced in the fat body cells of flies in the *PLA2* silencing group because the lipid droplets in the fat body were larger in the control group than in the *PLA2* silencing group (Fig. 5).

4. Discussion

In this study, we detected the expression profile of *PLA2* in *B. dorsalis* and found that the egg stage is one of the highest expression developmental stages of *PLA2*. This result was consistent with studies revealing that lipids are the main energy source for embryogenesis in insects (Beenakkers et al., 1980; Kinsella, 1966) because *PLA2* plays an important role in lipid digestion (Stanley, 2006).

We note that bacterial challenges stimulated significant increases in *PLA2* expression, and the highest up-regulation appeared in the fat body tissue. Silencing *PLA2* influenced the activation of the Toll/Imd signaling pathways in *B. dorsalis*, and also decreased the MTD after injection of *L. monocytogenes*. These results indicated that the *PLA2* gene is involved in the humoral immune response. Antimicrobial peptides are mainly produced by the fat body in *Drosophila* (Azeez et al., 2014). This is consistent with our results showing that *PLA2* increased most in the fat body after bacterial infection. PLA2 catalyzes the hydrolysis of phospholipids at the sn-2 position and usually releases arachidonic acid (Stanley, 2006). Arachidonic acid and other membrane phospholipids can generate a number of cell signaling molecules (Stanley and Kim, 2014), and phospholipids or phospholipid-derived mediators play important roles in regulating the activity of some proteins involved in cell signaling mechanisms within immune cells (Calder, 2001). The eicosanoids, which are oxygenated derivatives of arachidonic acid, are crucial signal moieties in the insect immune system (Stanleysamuelson et al., 1991). These results suggest that the *PLA2* gene mediates the humoral immune responses via influencing the release of fatty acids hydrolyzed from phospholipids.

PLA2 increased the most in the fat body after bacterial infection, and *PLA2* silencing reduced lipid storage in fat body cells. These results indicate that *PLA2* might take part in humoral immune responses by affecting the functions of fat body tissue in *B. dorsalis*. The most important hallmark of fat body cells is the presence of numerous lipid droplets (Arrese and Soulages, 2010). The PLA2 enzymes have recently been identified as key regulators of lipid droplet homeostasis (Guijas et al., 2014). The fat body of insects is a multi-functional center of metabolic activity and functions as lipid, carbohydrate and protein storage (Roma et al., 2010). The fat body is also the major site of transcription of the inducible antibacterial peptides (Hoffmann, 1995). Therefore, the phenotypic changes of the fat body after silencing *PLA2* in our study suggest that *PLA2* may influence the humoral immune responses by reducing lipid storage in fat body cells in *B. dorsalis*.

Acknowledgments

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