Bulletin of Entomological Research

cambridge.org/ber

Heat stress-induced expression of *Px-pdrg* and *Px-aspp2* in insecticide-resistant and -susceptible *Plutella xylostella*

Yu Wang, Jing Nan Wang, Xue Zhun Chen, Qi Xing Hu, Qi Qing Liu and Gang Wu

Key Laboratory of Biopesticide and Chemical Biology (Ministry of Education), Fujian Agriculture and Forestry University, Fuzhou, China

Research Paper

Cite this article: Wang Y, Wang JN, Chen XZ, Hu QX, Liu QQ, Wu G (2020). Heat stress-induced expression of *Px-pdrg* and *Px-aspp2* in insecticide-resistant and -susceptible *Plutella xylostella*. *Bulletin of Entomological Research* **110**, 177–184. https://doi.org/10.1017/S0007485319000543

Received: 29 August 2018
Revised: 25 March 2019
Accepted: 14 May 2019
First published online: 27 September 2019

Keywords:
Fitness cost; heat stress; *Plutella xylostella*; *Px-aspp2*; *Px-pdrg*

Author for correspondence:
Gang Wu, E-mail: newugang@163.com

Abstract

p53, DNA damage regulated gene (PDRG) and apoptosis-stimulating p53 protein 2 (ASPP2) are p53-related genes which can promote apoptosis. The full-length cDNA sequence of the *Px-pdrg* and *Px-aspp2* genes were characterized and their mRNA expression dynamics under heat stress were studied in diamondback moth (DBM) *Plutella xylostella* collected from Fuzhou, China. The full-length cDNA of *Px-pdrg and Px-aspp2* spans 721 and 4201 bp, containing 395 and 3216 bp of the open reading frame, which encode a putative protein comprising 130 and 1072 amino acids with a calculated molecular weight of 14.58 and 118.91 kDa, respectively. As compared to 25°C, both *Px-pdrg* and *Px-aspp2* were upregulated in chlorpyrifos-resistant (Rc) and -susceptible (Sm) strains of DBM adults and pupae under heat stress. In addition, Rc DBM showed a significantly higher expression level of *Px-pdrg* and *Px-aspp2* in contrast to Sm DBM. The results indicate that high temperature can significantly promote apoptosis process, especially in Rc-DBM. Significant fitness cost in Rc-DBM might be associated with drastically higher transcript abundance of *Px-pdrg and Px-aspp2* under the heat stress.

Introduction

Apoptosis is an important function of survival under environmental stress and survival pressure (Mosser *et al.*, 1997; Hebert *et al.*, 2009). The mechanisms of apoptosis network are highly complex, including two main apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway (Elmore, 2007). p53, a crucial regulator in apoptosis, is involved in the response to a range of stress types by regulating apoptosis, DNA repair, genetic stability, cell cycle arrest, and senescence (Laptenko and Prives, 2006). Many studies have shown that apoptosis is positively correlated with heat stress (Verheij *et al.*, 1996; Haimovitz-Friedman *et al.*, 1997) and plays a key role in normal development (Clem, 2001). In response to pressure signals, cytoplasmic p53 translocated rapidly to mitochondria, where it interacted with multidomain members of the pro- and anti-apoptotic bcl-2 family members to either inhibit or activate them (Vaseva and Moll, 2009).

p53 and DNA damage regulated gene (PDRG) are the genes which are associated with apoptosis and cell cycle regulation (Luo *et al.*, 2003; Jiang *et al.*, 2011; Wang *et al.*, 2014; Zhao *et al.*, 2017). It is known that PDRG appears as a specific marker in DNA damage and be selectively regulated by agents that induced genotoxic stress in vertebrates (Luo *et al.*, 2003; Jiang *et al.*, 2011). PDRG is differently regulated by crucial regulator p53 and genotoxic stress ultraviolet radiation (UV) (Luo *et al.*, 2004), and always strongly over-expresses in malignant tumor (Jiang *et al.*, 2009; Wang *et al.*, 2015). Exhaustion of PDRG in colon cancer can induce a decrease in cell multiplying (Paolo *et al.*, 2013). Treatment at 25°C results in aestivation, digestive tract atrophy, and degeneration and upregulates the expression of PDRG in sea cucumber *Apostichopus japonicus* (Wang and Yang, 2013).

Apoptosis-stimulating p53 protein 2 (ASPP2) is a member of tumor suppressor family ASPP (ASPPs) (Sullivan and Lu, 2007), being involved in the interaction between the ARD and the SH3 domain and the P53 (Cao *et al.*, 2004). Initially, ASPPs have been identified as the modulators of p53 transcriptional activity on pro-apoptotic DNA promoters. The proapoptotic members ASPP1 and ASPP2 stimulate p53-dependent apoptosis through induction of proapoptotic target genes, but the iASPP has the opposite function, and it can suppress the pro-apoptotic function of p53 (Samuels *et al.*, 2001; Bergamaschi *et al.*, 2003). ASPP2 is one of the most characteristic members of ASPP family which can promote apoptosis (Sullivan and Lu, 2007) and has central significance in cell growth and regulation of apoptosis (Vives *et al.*, 2006). ASPP2-mediated apoptosis could be inhibited by bcl-2 family proteins (a regulator in the mitochondrial apoptosis pathway) (Yang *et al.*, 1999). ASPP2 if found in the mitochondria,

CAMBRIDGE UNIVERSITY PRESS

suggesting that ASPP2 may play a role in promoting apoptosis via the mitochondrial apoptosis pathway (Takahashi *et al*., 2005).

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) is a worldwide cruciferous pest and has developed high insecticide resistance (Wu, 2002; Whalon *et al*., 2016). It is known that fitness cost from insecticide resistance has been determined in many insect species (Gassmann *et al*., 2009). However, the majority of studies in this field were conducted at the temperature which is conducive to the survival and reproduction of insects. Our previous results confirmed that high temperature could lead to significant fitness cost in chlorpyrifos-resistant (Rc) DBM. As compared to chlorpyrifos-susceptible (Sm) DBM, RcDBM showed significant fitness cost, including significantly decreased population growth trend index and fertility lower thermotolerance and fecundity (Zhang *et al*., 2015*a*, 2015*b*, 2017), higher hind-wing venation damages (Zhang *et al*., 2015*a*, 2015*b*) and higher reproductive cell apoptosis and oxidative stress (Zhang *et al*., 2017). The higher expression level of apoptosis-related genes in mitochondria apoptosis pathway was thought to be involved in the caused significant fitness cost by insecticide resistance in Rc-DBM (Zhuang *et al*., 2011, 2014, 2015*b*, 2017). However, the potential role of p53mediated apoptosis pathway is still unknown in DBM. In addition to mitochondria apoptosis pathway, p53 is thought to be the crucial regulator in apoptosis (Laptenko and Prives, 2006). p53-mediated apoptosis pathway is involved in the response to a range of stress types by regulating apoptosis, and PDRG and ASPP2 were verified to play an important role in p53-mediated apoptosis pathway in the vertebrate. The p53-mediated apoptosis pathway had been extensively investigated in *Homo sapiens* and *Drosophila melanogaster* (Susan *et al*., 2003; Brodsky *et al*., 2004; Laptenko and Prives, 2006; Titen and Golic, 2008). The important role of PDRG and ASPP2 in the p53-mediated apoptosis pathway had also been verified in vertebrata (Luo *et al*., 2003; Susan *et al*., 2003; Brodsky *et al*., 2004; Titen and Golic, 2008). Because apoptosis gene expression in mitochondria apoptosis pathway was involved in the fitness cost of Rc DBM by treating the pupae and adults of Rc or Sm DBM in different thermal stress groups (Zhang *et al*., 2015*b*, 2017), we speculate that p53-mediated apoptosis pathway might be involved in the fitness cost by insecticide resistance in Rc DBM. However, it could not be found that the p53-mediated apoptosis pathway was involved in the fitness cost caused by insecticide resistance. It was speculated that PDRG and ASPP2, an important role in p53-mediated apoptosis pathway in vertebrate, might be involved the apoptosis and fitness cost of Rc DBM because of different apoptosis profiles in Rc and Sm DBM. In this way, the expression profiles of PDRG and ASPP2 in Rc and Sm should be studied. Although cDNA sequences of PDRG and ASPP2 had been identified in some other insects in NCBI, the function and expression profiles of PDRG and ASPP2 in insects remain elusive. In the present study, the pupae and adults from the same insect strains (i.e., Rc and Sm DBM) were treated with the same way of thermal stress as described by Zhang *et al*. (2015*b*), and then identified of PDRG and ASPP2 genes and their mRNA expression under heat stress were explored to determine if the two genes in Rc and Sm DBM were related to the higher fitness cost in Rc DBM under heat stress.

Material and methods

Experimental insects

Resistant and sensitive strains of DBM were long-term reared in our laboratory. The Sm-strain is highly sensitive to chlorpyrifos whereas Rc-strain is highly resistant to this insecticide. Rc-strain showed 80 times resistance ratios to chlorpyrifos as high as Sm-strain. These strains were established from the population collected from Shangjie (34°480N, 113°180E) (Fuzhou, Fujian, China) and these two strains are maintained as Zhang *et al*. (2015*a*). No specific permissions were required for our collection of *P. xylostella*, because the scientists were welcome to collect the insect sample from the farmer's crucifer fields in order to control the pest insects. The field studies did not influence any endangered or protected species.

Cloning and nucleotide sequencing of Px-pdrg and Px-aspp2

Amplification of the initial fragments

Total RNAs were extracted from DBM adults or pupae using miniBEST universal RNA Extraction Kit (TaKaRa Bio Inc, Otsu, Japan). The first-strand cDNA was synthesized from total RNA with 500 ng by using PrimeScript™ RT reagent Kit (TaKaRa Bio Inc). The initial fragments of PDRG and ASPP2 (named as *Px-pdrg1-1*and *Px-aspp2-1* in DBM, respectively) were amplified by RT-PCR with the specific primers (table 1). These Primers were designed on the genetic information provided by the DBM Genome Database using Primer 5.0. PCR and the thermal cycling conditions were employed as follows: 94°C denaturation for 3 min, followed by 35 cycles of 94°C for 1 min, an annealing step at 56°C (56°C for PDRG; 54°C for ASPP2) for 30 s, and extension at 72°C for 2 min, finally followed by an extension step at 72°C for 7 min. In this step, two internal fragments of *Px-pdrg* and *Px-aspp2* with the length of 294 and 2174 bp were obtained.

Rapid amplification of cDNA ends (RACE) of PDRG and ASPP2

For 5′-and 3′-RACE, the first-strand cDNAs for the rapid amplification of cDNA ends (RACE) were synthesized with 1 μg total RNA according to the SMARTer® RACE 5′/3′ Kit User Manual (Takara Bio USA, Inc). The amplification and re-amplification nested specific primers for 3′- and 5′-RACE of PDRG and ASPP2 were used in our experiments (table 1), PCR reactions were run under the following conditions: 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 60°C for 30 min, and 72°C for 2 min 30 s, finally 72°C for 7 min. The three fragments (the initial fragments, the 5′-and 3′-RACE fragments) and the open reading frame (ORF) of *Px-pdrg* and *Px-aspp2* were cloned and sequenced by Shanghai Biosune Biotechnology Co, Ltd, Shanghai, China. Finally, these fragments were assembled to get the full cDNA sequence of the two genes.

Real-time quantitative PCR (qPCR)

Temperature shock

The pupae and adults of Rc or Sm DBM were pretreated in different thermal stress groups. During the hottest day of the period, the daily temperature in Fuzhou can reach 38–40°C for 6–8 h, 40–42°C for 2–3 h, and 42°C for 1–2 h in the fields. The treatment time and temperature were designed according to the temperature fluctuations during summer. Therefore, to imitate the real temperature fluctuations in the hottest season (July 10–August 10) in Fuzhou (Zhang *et al*., 2015*a*), these six groups were designed as follows: 38°C for 48 h, 40°C for 8 and 16 h, 42°C for 4 and 8 h, and 44°C −1 h. These treatments were set for. After the exposure of heat stress, the pupae and adults of

https://doi.org/10.1017/S0007485319000543 Published online by Cambridge University Press

Table 1. Sequences of primers used for cloning *Px-pdrg* and *Px-aspp2* cDNAs of DBM

Names of primers		Sequences of primers (5′–3′)	Tm (°C)	Isolated gene	Positions of forward and reverse prim
For initial fragment(s)					
DP-1F		5′-GAGAAGTTGGCTGAAGAA-3′	52.74	*Px-pdrg1-1*	305–320, 451–468
DP-1R		5′-TTTAGCAGACAGAGGTGC-3′	55.02		157–214, 599–616
2SP-1F		5′-TCAGAGCATCAGCCATAACC-3′	59.58	*Px-aspp2-1*	510–529, 2991–3010
2SP-1R		5′-CGTCCTCTTCACACTTCT 3′	59.85		819–836, 2684–2710
For RACE					
DP-3-1		3′-GTTGATGTTAGCACCTCTGTC	58.01	*Px-pdrg-3-1*	164–184, 589–609
DP-3-2		3′-TGAGGCTGACCAGAAACAAC	57.8		273–292, 481–500
DP-5-1		5′-TCACAAGTACAGATCCCACAG	55.75	*Px-pdrg-5-1*	324–344, 429–449
DP-5-2		5′-CACAAGTACAGATCCCACAGTAAC	60.26		325–348, 425–448
SP-3-1		3′-CTACTCAGGTCCAAAACGCCA	60.26	*Px-aspp2-3-1*	1071–1091, 2429–2499
SP-3-2		3′-CAATGTTCTCAGGCGGTCTAAGA	61.9		1209–1231, 2289–2311
SP-5-1		5′-CCCTTGATACTGGCTGTCCTGAA	61.9	*Px-aspp2-5-1*	704–726, 2794–2816
SP-5-2		5′-GACACGGGCTTTCCAAATCTTA	59.85		1400–1421, 2099–2120
UPM	L	5′-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3′			
	S	5′-CTAATACGACTCACTATAGGGC-3′			
NUP		5′-AAGCAGTGGTAACAACGCAGAGT-3′			
For ORF					
DP-C-1F		5′-TCTCATTCTACTCCATCTAG-3′	50.1	PDRG	
DP-C-1R		5′-CACTCCGAAGCTTATTAATA-3′	50.2		
SP-C-1F		5′ TTCAGGAACATACGCAAAGG 3′	56.3	ASPP2	
SP-C-1R		5′ TAAAACCTGCGGCGAACATA 3′	59.5		

Table 2. Sequences of primers used for qPCR of *Px-pdrg* and *Px-aspp2* of DBM

Primers	Sequences of primers (5′–3′)	Tm (°C)	Gene names	Positions of forward and reverse primers
PD-1F	5′-GCTTGAGGCTGACCAGAAAC-3′	59.9	*Px-pdrg-1*	478–497, 565–584
PD-1R	5′-GAACAGGTGGCTGCATCTCT-3′	59.9		
AS-1F	5′-AAGGAAGTGTCGACCTTAGTGTC-3′	60.2	*Px-aspp2*	2945–2967, 3026–3047
AS-1R	5′-TAGACCGCCTGAGAACATTGTC-3′	60.1		
B-1F	5′-GACCGACTACCTCATGAAGATCC-3′	61.2	*β-actin*	546–588, 623–645
B-1R	5′-GTAGCAGAGCTTCTCCTTGATGT-3′	60.1		
EF-1F	5′-TCGGAGTCATCAAGTCTGTCAAC-3′	60.2	*elongation factor 1*	1422–1444, 1549–1571
EF-1R	5′-CCTTTCGGTGAATGAAGTATCGC-3′	60.2		

Rc or Sm DBM were recovered at 25°C for 1 h before they were used in the following experiments.

Determination of mRNA expression

Total RNAs were extracted by TaKaRa miniBEST universal RNA Extraction Kit (TaKaRa Bio Inc) and cDNAs were synthesized with PrimeScript™ RT reagent Kit (Perfect Real Time) (TaKaRa Bio Inc). The expression level of our target genes was detected by real-time quantitative polymerase chain reaction (qPCR) using an Applied Biosystems 7500 Real-Time PCR System (AB, Life Technologies), and RT-qPCR were performed with TB Green™ Premix Ex Taq™ (Tli RNaseH Plus), Bulk (TaKaRa Bio Inc). The specific primers for qPCR analysis were listed in table 2. *β-actin* and *elongation factor* 1(EF-1) genes served as the internal reference genes. qPCR conditions were set as follows: 95°C for 30 s, 35 cycles of 95°C for 5 s, 60°C for 34 s, an additional step for measure the SYBR Green fluorescence at annealing temperature for 15 s. According to the threshold cycle (Ct) and equation of the standard curve, the expression level of *Px-pdrg* and *Px-aspp2* in different groups was normalized

https://doi.org/10.1017/S0007485319000543 Published online by Cambridge University Press

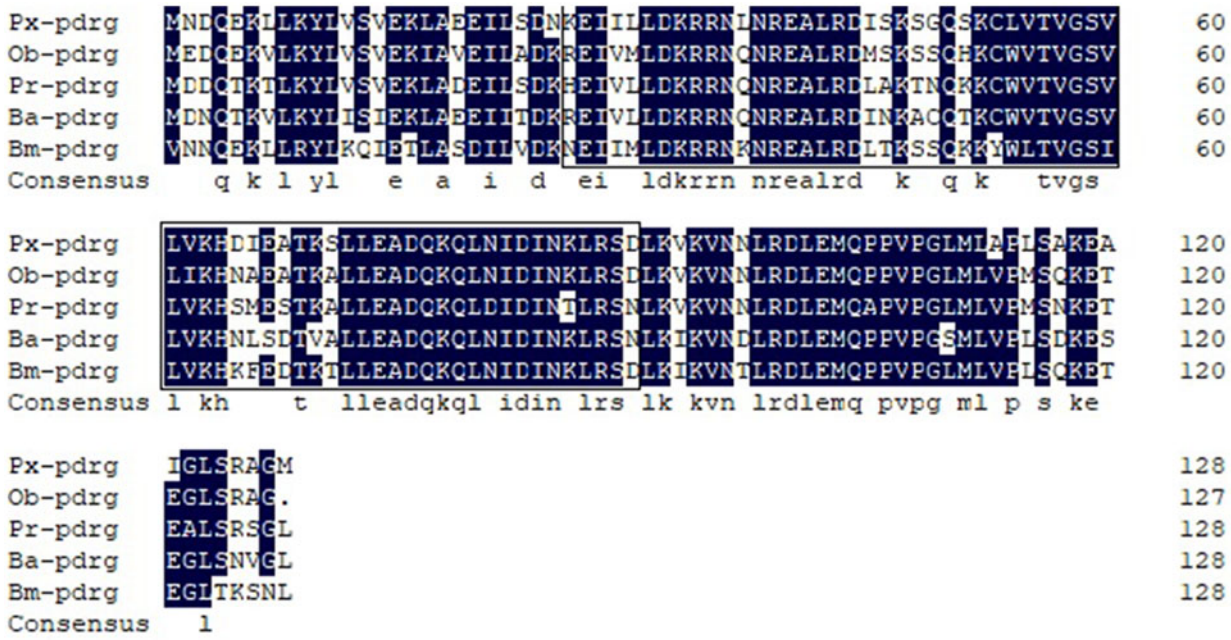

Figure 1. Multiple alignments of the deduced amino acid sequence of *Px-pdrg* with other known PDRG aligned by DNAMAN V6. Identical and 100% similar amino acids are labeled below, the dark areas also represent the same amino acids respectively. Black rectangles represent the phylogenetically BRLZ conserved domain. The species names and GenBank accession numbers are as follows: *Px-pdrg*: *Plutella xylostella* (MH727581); *Ob-pdrg*: *Operophtera brumata* (KOB77734.1); *Pr-pdrg*: *Pieris rapae* (XP_022128154.1); *Ba-pdrg*: *Bicyclus anynana* (XP_ 023934411.1); *Bm-pdrg*: *Bombyx mori* (XP_004928502.1).

to the reference genes (*EF-1* and *β-actin*) (Schmittgen and Livak, 2008; Zhang *et al.*, 2015*c*).

Statistical analysis

The significance in the expression level of our target genes across different groups was determined by probit analysis using a DPS data processing system (Tang and Feng, 1997).

Results

Cloning and sequence analysis of Px-pdrg *and* Px-aspp2

The full-length cDNA sequence of *Px-pdrg* (MH727581) and *Px-aspp2* (MH727582) is composed of 721 and 4201 bp nucleotides, respectively. The length of *Px-pdrg* (260–653 bp) and *Px-aspp2* (491–3710 bp) ORF sequence was 393 and 3209 bp, respectively. The polypeptide, being encoded by *Px-pdrg* and *Px-aspp2*, was consisted of 130 and 1072 amino acids. Their calculated molecular weight is 14.58 and 118.91 kDa, the theoretical pI (isoelectric point) for them is 8.69 and 7.33, respectively. The SMART (http://smart.embl-heidelberg.de/) analysis show that the ORF of *Px-pdrg* contains a BRLZ (basic region leucine zipper) domain (position 26–93 aa) (fig. 1 and fig. S1). Px-aspp2 has the typical conservative characteristics of the ASPP family, possessing a ARD (ankyrin repeat domain) domain with four ANK structural domains (position 830–976 aa), a SH3 domain (position 1007–1065 aa) and a coiled coli region (position 57–145 aa) (fig. 2 and fig. S2).

Px-pdrga and Px-aspp2 have a higher identity with the reported orthologs from Lepidoptera. For instance, *Px-pdrg* has 82, 76, 73, and 71% identity with *Operophtera brumata* (KOB77734.1), *Pieris rapae* (XP_022128154.1), *Ba-pdrg*, *Bicyclus anynana* (XP_ 023934411.1) (fig. 3). When compared to those proteins from hymenoptera, the amino acid sequence of *Px-pdrg* exhibits 55, 54, and 54% identity with *Camponotus floridanus* (XP_011262329.1), *Harpegnathos saltator* (XP_011140755.1), and *Atta colombica* (KYM88266.1). As compared to those of vertebrate, *Px-pdrg* only has 38.76 and 43% identity with *Homo sapiens* (NP_110442.1) and *Mus musculus* (EDL06 006.1) (fig. 3). *Px-aspp2* has depicted 76, 77, 76, and 73% identity with *Bicyclus anynana* (XP_023941372.1), *Spodoptera litura* (XP_022827177.1), *Helicoverpa armigera* (XP_021193 800.1), and *Bombyx mori* (XP_012547617.1) (fig. 4). In contrast to Hymenopteran, *Px-aspp2* has 67 and 65% identity with *Habropoda laboriosa* (KOC70575.1) and *Bombus terrestris* (XP_012167971.1). For Coleopteran, *Px-aspp2* shows 40 and 49% identity with *Cimex lectularius-X5* (XP_ 0142425 20.1) and *Anoplophora glabripennis* (XP_018576232.1). *Px-aspp2* only has 56.3% identity with *Homo sapiens* (AK294432.1) (fig. 4). The phylogenetic analysis showed that *Px-pdrg* and *Px-aspp2* belonged to PDRG and ASPP family, respectively (figs 1–4).

Heat stress-induced expression of Px-pdrg *and* Px-aspp2

As shown in fig. 5, the basic expression level of *Px-pdrg* and *Px-aspp2* in pupae or adults was relatively lower in Rc or Sm DBM at 25°C. Their expression level in adult was significantly higher than those in pupae under the exposure of heat shock (at 42 and 44°C), and the greatest transcript abundance of *Px-pdrg* was found in pupae and adults at 42°C-8 h. The highest expression level of *Px-aspp2* was detected in pupae at 40°C-16 h, or in adult at 42°C-8 h. The highest thermal excitation effect of *Px-pdrg* was found in pupae at 42°C-8 h and that of *Px-aspp2* in pupae and adults. The two genes were drastically induced to express in Rc and Sm DBM adults or pupae after being exposed to heat stress, and the expression level of *Px-pdrg* in adult was

https://doi.org/10.1017/S0007485319000543 Published online by Cambridge University Press

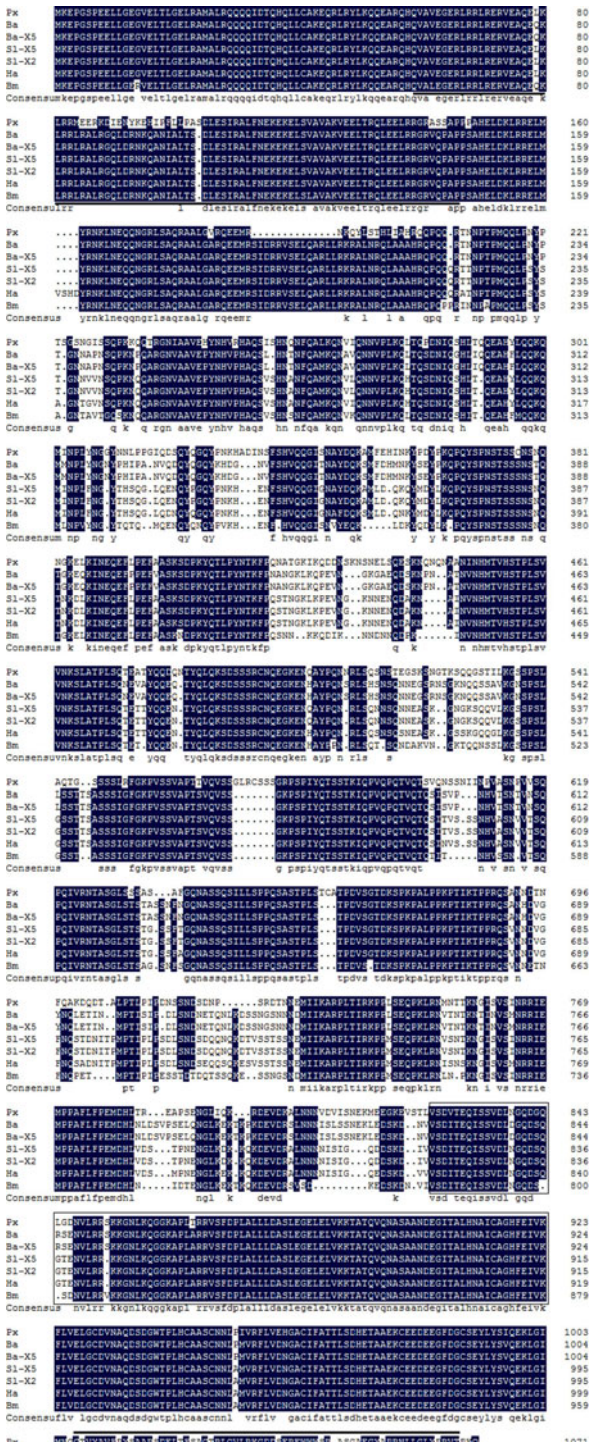

Figure 2. Multiple alignments of *Px-aspp2* amino acid sequence in DBM with other ASPP2 in different species aligned by DNAMAN V6. The same amino acid area is covered in dark,100% of similar sites are indicated with amino acids at below, respectively. The underline marks coiled coli region(position57–145). Black rectangles represent ARD (ankyrinrepeat domain). The overline represent SH3 (Src-homolog y3 domain), the species names and GenBank accession numbers are as follows: *Px*: *Plutella xylostella* (MH727582); *Ba*: *Bicyclus anynana* (XP_023941372.1); *Ba-X5*: *Bicyclus anynana* (XP_02394 1371.1); *Sl-X5*: *Spodoptera litura* (XP_022827177.1); *Sl-X2*: *Spodoptera litura* (XP_022827174.1); *Ha*: *Helicoverpa armigera* (XP_021193 800.1); *Bm*: *Bombyx mori* (XP_012547617.1).

higher than that in pupae. Although the expression level of the two genes was up-regulated, the up-regulation expression magnitude of the two genes in DBM Rc pupae and adults was higher than that of Sm ones.

Discussion

The full cDNA sequence of *Px-pdrg* and *Px-aspp2* were identified from *P. xylostella*. Although the deduced amino acid sequence has some differentiations as compared to those of vertebrates, two proteins, being encoded by the two genes, showed highly conserved relationships with these orthologs.

In human, PDRG gene locates at the long arm of chromosome 20 and encodes 133 amino acids protein (Luo *et al.*, 2003; Jiang *et al.*, 2011). In this study, *Px-pdrg* spans 721 bp, contained 395 bp of the ORF, which produced a putative protein comprising 130 amino acids, have a conversed domain BRLZ and shows higher identity with the ortholog of other insect species. The ASPPs protein family consists of three members, ASPP1, ASPP2 and ASPP (iASPP) (Samuels-Lev *et al.*, 2001; Trigiante and Lu, 2006). ASPP2 shares highly conserved C-terminus domains: an ankyrin repeat domain (ARD) and an Src-homology 3 (SH3) domain (Slee and Lu, 2003). In this study, Px-aspp2 contains an ARD (ankyrin repeat domain) domain with four ANK structural domains and an SH3 domain (figs 1 and 2). The sequences of the two genes in several insect species have been registered in NCBI. According to the Blast results, *Px-pdrg* exhibits 71–82% similarity with those of other Lepidopteran insects (such as *Operophtera brumata*, *Pieris rapae*, *Bicyclus anynana*, and *Bombyx mori*), 54–55% to Hymenoptera insects (such as *Camponotus floridanus*, *Harpegnathos saltator*, and *Atta colombica*), and 42.3% similarity to *Mus musculus*. The amino acid sequence of *Px-aspp2* possesses 73–76% similarity as compared to other Lepidopteran insects (such as *Bicyclus anynana*, *Spodoptera litura*, *Helicoverpa armigera*, and *Bombyx mori)*, and 65–67% to Hymenoptera insects (such as *Habropoda laboriosa* and *Bombus terrestris*), and 40 and 49% to Coleopteran (such as *Cimex lectularius* and *Anoplophora glabripennis)*, 56.3% to *H. sapiens*. The phylogenetic analysis showed that *Px-pdrg* and *Px-aspp2* have the highest identity to those of Lepidopteran (figs 3 and 4). Moreover, Px-pdrg and Px-aspp2 have highly conserved functional domains that have been reported in other species. Therefore, these results indicate that *Px-pdrg* and *Px-aspp2* are the member of the PDRG and ASPPs family, respectively.

PDRG and ASPP2 are p53-related genes that play the important role in apoptosis (Jiang *et al.*, 2011), Moreover, PDRG can be upregulated to promote apoptosis under the stress of genotoxic such as ultraviolet radiation (Luo *et al.*, 2003). ASPP2 expression levels are generally increased in tumor cells and cells with unstable replication. High temperature is a common environmental stress which can promote apoptosis in a variety of organisms (Juan *et al.*, 2006). In DBM, the study of thermal stress had been studied (Shirai, 2000). As compared to those in Sm DBM, the life history parameters (Zhang *et al.*, 2015*a*) and physiological protection (Zhang *et al.*, 2015*a*, 2015*b*, 2015*c*) are inhibited significantly, while the expression levels of apoptosis and the damages of reproduction cell associated genes are increased significantly under heat stress in Rc DBM.

The present study aimed at revealing the effects of high temperature on the mRNA expression of the apoptosis related genes (*Px-pdrg* and *Px-aspp2*) in insecticide-resistant

https://doi.org/10.1017/S0007485319000543 Published online by Cambridge University Press

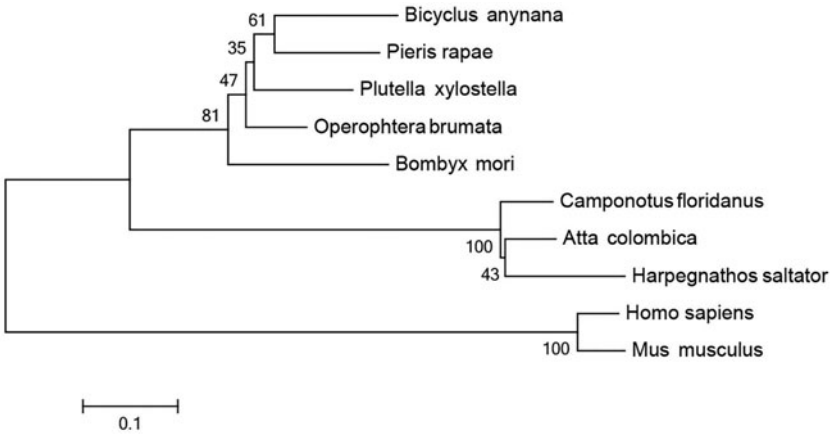

Figure 3. Based on amino acid sequence encoded by *Px-pdrg*, NJ phylogenetic tree was constructed by MEGA 6. 0 software. The bootstrap values numbers above the branches (1000 replicates). *Px-pdrg-1* protein family members used in phylogenetic analysis were as follows: *Bicyclus anynana* (XP_023934411.1); *Pieris rapae* (XP_022128154.1); *Plutella xylostella* (MH727581); *Operophtera brumata* (KOB77734.1); *Bombyx mori* (XP_004928502.1); *Camponotus floridanus* (XP_011262329.1); *Atta colombica* (KYM88266.1); *Harpegnathos saltator* (XP_011140755.1); *Homo sapiens* (NP_110442.1); *Mus musculus* (EDL06006. 1).

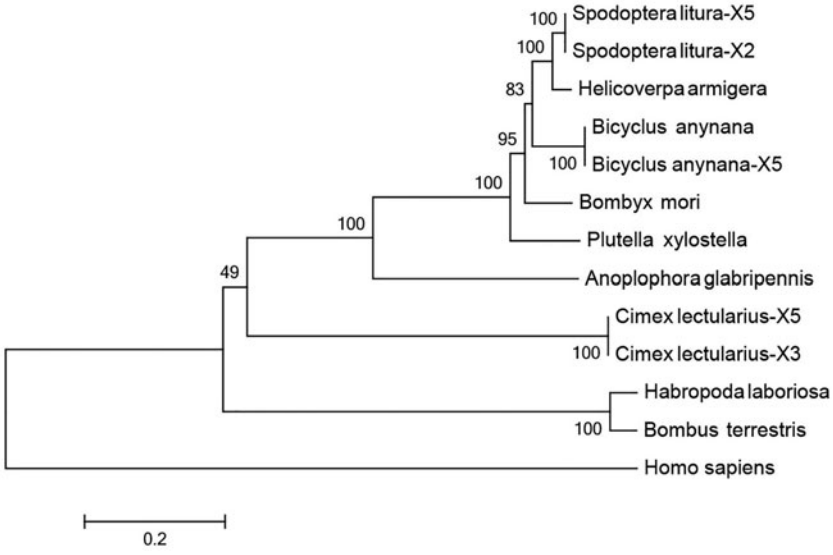

Figure 4. NJ phylogenetic tree based on amino acid sequence encoded by *Px-aspp2* and made by MEGA 6.0. The number above the branch is the bootstrap values at (1000 replicates), The following ASPP2 proteins family members were used in the phylogenetic analysis: *Spodoptera litura-X5* (XP_022827177.1); *Spodoptera litura-X2* (XP_022827174.1); *Helicoverpa armigera* (XP_021193800.1); *Bicyclus anynana* (XP_023941372.1); *Bicyclus anynana-X5* (XP_023941371.1); *Bombyx mori* (XP_01254 7617.1); *Plutella xylostella* (MH727582); *Anoplophora glabripennis* (XP_018576232.1); *Cimex lectularius-X5* (XP_ 0142425 20.1); *Cimex lectularius-X3* (XP_014242517.1); *Helicoverpa armigera* (XP_021193800.1); *Bombus terrestris* (XP_012167971.1); *Homo sapiens* (AK294432.1).

and -susceptible DBM. We found the expression levels of *Px-pdrg* and *Px-aspp2* genes in both Rc and Sm DBM were low at normal temperature (25°C), but heat stress upregulated their expression levels significantly. It has been reported that the apoptosis is closely related to the up-regulation of PDRG and ASPP2 genes (Luo *et al.*, 2004). The similar phenomenon was also observed in the present study. For instance, the highest thermal excitation effect of *Px-pdrg* was found in pupae under the exposure of 42° C-8 h and that of *Px-aspp2* in pupae and adults. In general, higher up-regulation expression levels of the two genes were found in Rc DBM. The fitness cost in life-history, behavior, and physiological traits in resistant insect species is a general tendency which has been determined in many insect species. However, these experiments were carried out under a suitable temperature (Gassmann *et al.*, 2009; Zhang *et al.*, 2015*a*). The higher expression level of caspase-7 in mitochondria apoptosis pathway was found in insecticide-resistant DBM under heat stress (Zhuang *et al.*, 2011, 2014, 2015*b*, 2017). The significantly higher expression level of *Px-pdrg* and *Px-aspp2* in p53-mediated apoptosis pathway was firstly found in DBM after being exposed to heat stress, indicating the higher fitness cost in insecticide-resistant insects. In insects, energy and resource are often allocated among different physiological activities to obtain optimal fitness. Physiological and genetic mechanisms to deal with toxic pesticides, sometimes these processes lead to the excessive expression of detoxifying enzymes in tissues or inducing mutations in target sites (Kliot and Ghanim, 2012). The fitness costs in insecticide-resistant insects are often from shutting down some normal cell functions during the stress-induced responses. It is suggested that the expression level of genes in each species and populations was the trade-off between benefits and costs (Kristensen *et al.*, 2008; Zhang *et al.*, 2015*b*). Significant fitness cost, including lower thermotolerance (Zhang *et al.*, 2015*a*) and higher damage in development of wing veins (Zhang *et al.*, 2015*b*) under heat stress in Rc DBM under heat stress, i.e., might be associated with their significantly higher expressions of apoptosis gene, such as caspase-7, Apaf-1, and cytochrome c gene (Zhuang *et al.*, 2011, 2014, 2015*b*, 2017), lower hsps gene expression (Zhang *et al.*, 2015*c*), and significantly higher transcript abundance of *Px-pdrg* and *Px-aspp2* in this study. The stimulation of effector caspases in the mitochondrial pathway (Zhuang *et al.*, 2011; Zhang *et al.*, 2014, 2015*b*, 2017) is closely related to the apoptosis network. Meanwhile, PDRG and ASPP2 are also important genes to promote apoptosis (Luo *et al.*, 2003;

https://doi.org/10.1017/S0007485319000543 Published online by Cambridge University Press

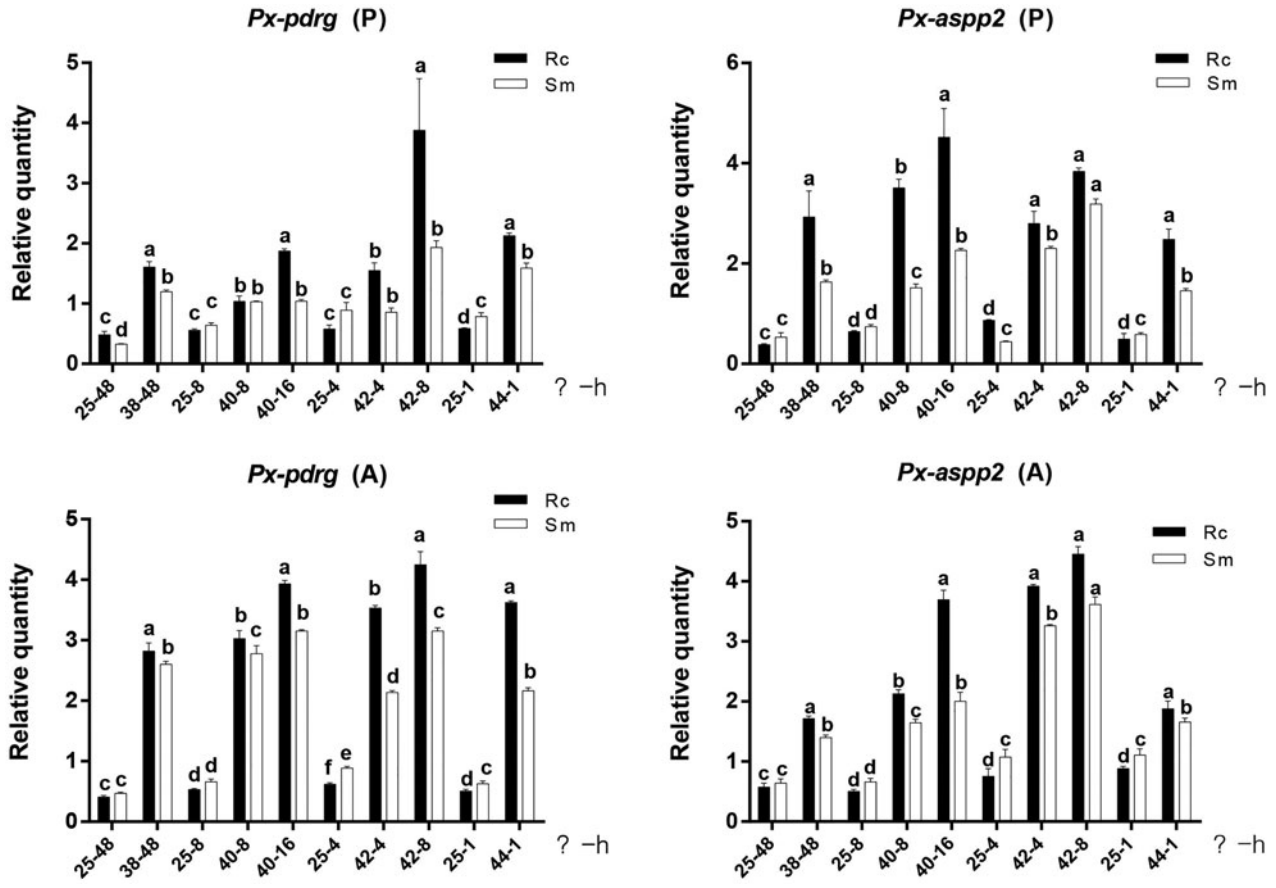

Figure 5. The relative expression levels of *Px-pdrg* and *Px-aspp2* in R_C (black) and S_C (white) under heat stress. *Px-pdrg* (P) and *Px-aspp2* (P) = pupae, *Px-pdrg* (A) and *Px-aspp2* (A) = adults. Temperature treatment included 38°C-48 h; 40°C-8,16 h; 42°C-4,8 h; and 44°C-1 h, respectively. Each bar represents the average of three independent experiments. The lower-case letters indicate a significant difference between Rc and Sm DBM (Duncan's-test, $P \leq 0.05$).

Susan *et al.*, 2003; Brodsky *et al.*, 2004; Titen and Golic, 2008). The detailed function of *Px-pdrg* and *Px-aspp2* in the apoptosis signaling of DBM individuals under heat stress needs further extensive investigations.

The R_C DBM insects commonly established in the field, and its required resistance is not only evolved by insecticide screening, but also by high temperature stress which can induce the apoptosis. Our findings indicate the R_C DBM insects have higher transcript abundance of apoptosis-related genes and greater fitness costs. Consequently, fluctuations in temperature can change the development of insecticide resistance in DBM. That is, the population trend of resistant DBM at different temperatures will alter correspondingly due to the different degree of apoptosis. It is important that fitness cost caused by insecticide resistance should be considered to maximize the effect of insecticides and minimize costs and residues of controlling insects when the insect management program is designed.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485319000543.

Acknowledgements. This study was financially supported by the National Natural Science Foundation of China (NNSFC) (31272049) and Key foundation of technology project of Fujian Province (2014N0003).

Author contributions. Conceived the experiments, analyzed the data, generated the tables, produced the figures, and wrote the manuscript: GW. Performed the experiments: YW, JNW, XZC, QXH, QQL, GW.

Conflict of interests. None.

References

Bergamaschi D, Samuels Y, O'Neil NJ, Trigiante G, Crook T, Hsieh JK, O'Connor DJ, Zhong S, Campargue I and Tomlison ML (2003) iASPP oncoprotein is a key inhibitor of p53 conserved from worm to human. *Nature Genetics* **33**, 162–167.

Brodsky MH, Weinert BT, Tsang G, Rong YS, Mcginnis NM, Golic KG, Rio DC and Rubin GM (2004) Drosophila melanogaster mnk/chk2 and p53 regulate multiple DNA repair and apoptotic pathways following DNA damage. *Molecular & Cellular Biology* **24**, 1219–1231.

Cao Y, Hamada T, Matsui T, Date T and Iwabuchi K (2004) Hepatitis C virus core protein interacts with p53-binding protein, 53BP2/Bbp/ASPP2, and inhibits p53-mediated apoptosis. *Biochemical and Biophysical Research Communications* **315**, 788–795.

Clem RJ (2001) Baculoviruses and apoptosis: the good, the bad, and the ugly. *Cell Death & Differentiation* **8**, 137–143.

Elmore S (2007) Apoptosis: a review of programmed cell death. *Toxicologic Pathology* **35**, 495–516.

Gassmann A, Carriere Y and Tabashnik B (2009) Fitness costs of insect resistance to bacillus thuringiensis. *Annual Review of Entomology* **54**, 147.

Haimovitz-Friedman A, Kolesnick RN and Fuks Z (1997) Ceramide signaling in apoptosis. *British Medical Bulletin* **53**, 539.

Hebert CG, Valdes JJ and Bentley WE (2009) Investigating apoptosis: characterization and analysis of trichoplusia ni-caspase-1 through overexpression and rnai mediated silencing. *Insect Biochemistry & Molecular Biology* **39**, 113.

Jiang S, Qiu L, Zhou F, Huang J, Guo Y and Yang K (2009) Molecular cloning and expression analysis of a heat shock protein (hsp90) gene from black tiger shrimp (penaeus monodon). *Molecular Biology Reports* **36**, 127–134.

Jiang L, Luo X, Shi J, Sun H, Sun Q, Sheikh MS and Huang Y (2011) Pdrg1, a novel tumor marker for multiple malignancies that is selectively regulated by genotoxic stress. *Cancer Biology & Therapy* **11**, 567–573.

Juan DU, He-Shuang DI, Guo L, Zhong-Hao LI and Cai YF (2006) The effect of high temperature on mammary epithelial cells proliferation and apoptosis. *Acta Zoologica Sinica* **52**, 959–965.

Kliot A and Ghanim M (2012) Fitness costs associated with insecticide resistance. *Pest Management Science* **68**, 1431–1437.

Kristensen TN, Hoffmann AA, Overgaard J, Sørensen JG, Hallas R and Loeschcke V (2008) Costs and benefits of cold acclimation in field-released *Drosophila*. *PNAS* **105**, 216–221.

Laptenko O and Prives C (2006) Transcriptional regulation by p53: one protein, many possibilities. *Cell Death & Differentiation* **13**, 951–961.

Luo X, Huang Y and Sheikh MS (2003) Cloning and characterization of a novel gene pdrg that is differentially regulated by p53 and ultraviolet radiation. *Oncogene* **22**, 7247–7257.

Luo X, Huang Y and Sheikh MS (2004) Pdrg, a novel p53 and DNA damage-regulated gene. *Cancer Research* **7**, 64–75.

Mosser DD, Caron AW, Bourget L, Denis-Larose C and Massie B (1997) Role of the human heat shock protein hsp70 in protection against stress-induced apoptosis. *Molecular & Cellular Biology* **17**, 5317–5327.

Paolo M, Savas JN, Susan H, Nabil D, Iii YJR and Logan SK (2013) Analysis of uri nuclear interaction with rpb5 and components of the r2tp/prefoldin-like complex. *PLoS ONE* **8**, e63879.

Samuels-Lev Y, O'Connor DJ, Bergamaschi D, Trigiante G, Hsieh JK, Zhong S, Campargue I, Naumovski L, Crook T and Lu X (2001) Aspp proteins specifically stimulate the apoptotic function of p53. *Molecular Cell* **8**, 781.

Schmittgen TD and Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. *Nature Protocols* **3**, 1101–1108.

Shirai Y (2000) Temperature tolerance of the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae) in tropical and temperate regions of Asia. *Bulletin of Entomological Research* **90**, 357–364.

Slee EA and Lu X (2003) The aspp family: deciding between life and death after DNA damage. *Toxicology Letters* **139**, 81.

Sullivan A and Lu X (2007) Aspp: a new family of oncogenes and tumour suppressor genes. *British Journal of Cancer* **96**, 196–200.

Susan H, Michael B, Zehavit G and Ygal H (2003) Apoptosis – the p53 network. *Journal of Cell Science* **116**, 4077–4085.

Takahashi N, Kobayashi S, Kajino S, Imai K, Tomoda K, Shimizu S and Okamoto T (2005) Inhibition of the 53bp2s-mediated apoptosis by nuclear factor κb and bcl-2 family proteins. *Genes to Cells* **10**, 803–811.

Tang QY and Feng MG (1997) Practical statistics and DPS data processing system. In Tang QY and Feng MG (eds), *DPS Data Processing System for Practical Statistics*. Beijing, China: China Agricultural Press, pp. 188–195.

Titen SWA and Golic KG (2008) Telomere loss provokes multiple pathways to apoptosis and produces genomic instability in drosophila melanogaster. *Genetics* **180**, 1821.

Trigiante G and Lu X (2006) Aspp [corrected] and cancer. *Nature Reviews Cancer* **6**, 217–226.

Vaseva AV and Moll UM (2009) The mitochondrial p53 pathway. *Biochimica et Biophysica Acta* **1787**, 414–420.

Verheij M, Bose R, Lin XH, Yao B, Jarvis WD, Grant S, Birrer MJ, Szabo E, Zon LI and Kyriakis JM (1996) Requirement for ceramide-initiated sapk/jnk signalling in stress-induced apoptosis. *Nature* **380**, 75–79.

Vives V, Su J, Zhong S, Ratnayaka I, Slee E, Goldin R and Lu X (2006) Aspp2 is a haploinsufficient tumor suppressor that cooperates with p53 to suppress tumor growth. *Genes and Development* **20**, 1262–1267.

Wang TM and Yang HS (2013) Cloning and characterization of pdrg gene from sea cucumber *Apostichopus japonicus* and the expression in intestine during aestivation. *Marine Sciences* **37**, 1–9.

Wang DQ, Wang K, Yan DW, Liu J, Wang B, Li MX, Wang XW, Liu J, Peng ZH and Li GX (2014) Ciz1 is a novel predictor of survival in human colon cancer. *Experimental Biology & Medicine* **239**, 862–870.

Wang J, Zhang X, Wang L, Yang Y, Dong Z, Wang H, Du L and Wang C (2015) Microrna-214 suppresses oncogenesis and exerts impact on prognosis by targeting pdrg1 in bladder cancer. *PLoS ONE* **10**, e0118086.

Whalon M, Motasanchez D, Hollingworth D and Duynslager L and Arthropod pesticide resistance database (APRD) (2016) Arthropod pesticide resistance database, Michigan State University, East Lansing. Available at http://www.pesticideresistance.com/index.php (Accessed 22 January 2016).

Wu G (2002) Field monitor of insecticide resistance and toxicological mechanism in *Plutella xylostella* (l.). *Journal of Plant Protection* **29**, 351–355.

Yang JP, Hori M, Takahashi N, Kawabe T, Kato H and Okamoto T (1999) Nf-kappab subunit p65 binds to 53bp2 and inhibits cell death induced by 53bp2. *Oncogene* **18**, 5177–5186.

Zhang LJ, Huang JF, Chen F, Yu ZY, Wu ZL and Wu G (2014) Identifications of cytochrome c and apaf-1 and their mRNA expressions under heat stress in insecticide-susceptible and -resistant *Plutella xylostella* (Lepidoptera: Plutellidae). *European Journal of Entomology* **111**, 457–468.

Zhang LJ, Jing YP, Li XH, Li CW, Bourguet D and Wu G (2015*a*) Temperature-sensitive fitness cost of insecticide resistance in Chinese populations of the diamondback moth *Plutella xylostella*. *Molecular Ecology* **24**, 1611–1627.

Zhang LJ, Wu ZL, Wang KF, Liu Q, Zhuang HM and Wu G (2015*b*) Trade-off between thermal tolerance and insecticide resistance in *Plutella xylostella*. *Ecology and Evolution* **5**, 515.

Zhang LJ, Wang KF, Jing YP, Zhuang HM and Wu G (2015*c*) Identification of heat shock protein genes hsp70s and hsc70 and their associated mRNA expression under heat stress in insecticide-resistant and susceptible diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae). *European Journal of Entomology* **112**, 215–226.

Zhang LJ, Chen JL, Yang BL, Kong XG, Bourguet D and Wu G (2017) Thermotolerance, oxidative stress, apoptosis, heat-shock proteins and damages to reproductive cells of insecticide-susceptible and -resistant strains of the diamondback moth *Plutella xylostella*. *Bulletin of Entomogical Research* **107**, 513–526.

Zhao C, Dai W and Qiu L (2017) Molecular cloning, characterization and expression analysis of a novelpdrg1 gene from black tiger shrimp (penaeus-monodon). *Genetics & Molecular Biology* **40**, 93–103.

Zhuang HM, Wang KF, Miyata T, Wu ZJ, Wu G and Xie LH (2011) Identification and expression of caspase-1 gene under heat stress in insecticide-susceptible and -resistant *Plutella xylostella*, (Lepidoptera: Plutellidae). *Molecular Biology Reports* **38**, 2529–2539.