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NADPH-cytochrome P450 reductase involved in the lambda-cyhalothrin susceptibility on the green mirid bug *Apolygus lucorum*

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Abstract

NADPH-cytochrome P450 reductase (CPR) is crucial for the detoxification process catalysed by cytochrome P450, which targets various exogenous xenobiotics, as well as pesticides. In our research, we successfully obtained the complete cDNA sequence of *Apolygus lucorum*'s CPR (*AlCPR*) using reverse transcription PCR along with rapid amplification of cDNA ends technology. Bioinformatics analysis exhibited that the inferred amino acid sequence of AlCPR is characteristic of standard CPRs, featuring an N-terminal membrane anchor and three conserved FMN, FAD and NADP binding sites. Phylogenetic result revealed that AlCPR was positioned within the Hemiptera cluster, showing a close evolutionary relationship with the CPR of *Cimex lectularius*. The real-time quantitative PCR results demonstrated widespread expression of *AlCPR* across various life stages and tissues of *A. lucorum*, with the most prominent expression in adults and the abdominal region. Injecting double-stranded RNA of *AlCPR* only significantly increased the lambda-cyhalothrin susceptibility in lambda-cyhalothrin-resistant strain rather than the susceptible strain. These findings suggest a potential link between AlCPR and the P450-dependent defence mechanism against lambda-cyhalothrin in *A. lucorum*.

Introduction

The green mirid bug *Apolygus lucorum* is a highly destructive omnivorous pest, and in China it inflicts a broad spectrum of damage across an array of economically significant crops, including cotton, vegetables, grains and fruit orchards (Lu *et al.*, 2010; Tan *et al.*, 2012). Lu *et al.* (2024) summarised the integrated mirid management strategies in China's cotton regions and pointed out farmers getting accustomed to using chemical insecticides for mirid control. The frequent usage of insecticides has unavoidably caused resistance in some *A. lucorum* field populations for recent years (Zhen and Gao, 2016; Zhen *et al.*, 2016; Zuo *et al.*, 2019; Wang *et al.*, 2020). More studies suggested that cytochrome P450 (CYPs) were involved in multiple insecticide resistance of *A. lucorum* (Zhen *et al.*, 2018; Wu *et al.*, 2019) and other Hemipteran pest (Ding *et al.*, 2013; Elzaki *et al.*, 2017; Wu *et al.*, 2018). For instance, Zhen *et al.* (2018) reported that various cytochrome P450 genes such as six *CYP6* genes (*CYP6HM1*, *CYP6HM2*, *CYP6JB1*, *CYP6JB2*, *CYP6JC1*, *CYP6X2* and *CYP395H1*) were upregulated in lambda-cyhalothrin-resistant strain of *A. lucorum*. Wu *et al.* (2019) pointed out that the *CYP395G1* overproduction appeared to be related to dinotefuran resistance in *A. lucorum*. Ding *et al.* (2013) proved that the induced CYP6AY1 was probably associated with the imidacloprid resistance in *Nilaparvata lugensto*. Elzaki *et al.* (2017) demonstrated that CYP353D1v2 could metabolise imidacloprid in *Laodelphax striatellus*, and Wu *et al.* (2018) documented that silencing *CYP6CY14* significantly enhanced the susceptibility to thiamethoxam in resistant aphids of *Aphis gossypii*.

As an important enzyme, NADPH-cytochrome P450 reductase (CPR) (one representative of EC 1.6.2.4) existed in all organisms and participated in a series of biochemical reactions catalysed via cytochrome P450 (CYPs), which served as the electron transfer partner from nicotinamide adenine dinucleotide phosphate (NADPH) to the central haeme group in the CYPs (Feyereisen, 1999; Paine *et al.*, 2005). Up to now, the identification of insect CPRs has been confirmed in over 30 species. Among these characterised CPRs, some had been verified to be involved in P450-mediated metabolism resistance in medical or agricultural pests, such as two medical pests the mosquito *Anopheles gambiae* (Lycett *et al.*, 2006) and the bed bug *C. lectularius* (Zhu *et al.*, 2012), and 12 agricultural pests including one Orthoptera pest *Locusta migratoria* known as the oriental migratory locust (Zhang *et al.*, 2017), one pest mite *Tetranychus cinnabarinus* with common name the carmine spider mite (Shi *et al.*, 2015), six Hemiptera pests the rice brown planthopper *N. lugens* (Liu *et al.*, 2015a),

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the small brown planthopper *L. striatellus* (Zhang *et al.*, 2016), the brown citrus aphid *Aphis (Toxoptera) citricidus* (Kirkaldy) (Jing *et al.*, 2018), the cotton aphid *Ap. gossypii* (Tang *et al.*, 2023), the pea aphid *Acyrthosiphon pisum* (Qiao *et al.*, 2022), the tobacco whitefly *Bemisia tabaci* Q (He *et al.*, 2020), one Diptera pest, the oriental fruit fly *Bactrocera dorsalis* (Huang *et al.*, 2015), and three Lepidoptera pests the cotton bollworm *Helicoverpa armigera* (Tang *et al.*, 2012; Zhao *et al.*, 2014), the beet armyworm *Spodoptera exigua* (Zhao *et al.*, 2015) and the tobacco cutworm *Spodoptera litura* (Ji *et al.*, 2019; Shi *et al.*, 2021). However, the characterisation and role of CPR in the resistance development in *A. lucorum* was scarce.

In our study, the basic characterisation of CPR from *A. lucorum* (*AlCPR*) was determined. The quantitative PCR (qPCR) method was utilised to determine the gene expression patterns across various developmental times and body parts. The significantly increased sensitivity against lambda-cyhalothrin was only observed in the lambda-cyhalothrin-resistant *A. lucorum* strain (LcR) when knockdowning *AlCPR*. Our study would provide solid foundations for further elucidation of the CPR function in the CYP-mediated lambda-cyhalothrin resistance in *A. lucorum*.

Materials and methods

Insect cultures

The insect *A. lucorum* was nurtured on a diet of *Phaseolus vulgaris* within ventilated plastic containers measuring 20 cm by 15 cm by 10 cm. They were maintained under the conditions as follows: temperature 26 ± 1°C, relative humidity 60 ± 5% and photoperiod 16L: 8D. Additionally, adults were added 10% honey. Two strains, SS (susceptible to lambda-cyhalothrin) and LcR (resistant to lambda-cyhalothrin, with resistance ratio >7.8-fold), were developed in our laboratory, and the same strains with the previous study (Zhen *et al.*, 2018). The susceptible (SS) strain was sourced from the transgenic *Bt* cotton fields at the Langfang Experimental Station, located in Hebei Province, China, with geographical coordinates of 39.517966 N latitude and 116.666811 E longitude in year 2008 and then raised in our laboratory without insecticide exposure. The LcR strain was obtained by the same population with lambda-cyhalothrin selection for 11 generations.

RNA isolation and cDNA preparation

Adult specimens of *A. lucorum* were prepared for total RNA isolation with TRIzol reagent supplied by Invitrogen, Carlsbad, CA as described by the manufacturer's instruction. A NAS-99 spectrophotometer from ACTGene was employed for assessing the RNA integrity and concentration. The synthesis of first-strand complementary DNA (cDNA) for qPCR was facilitated by employing the PrimeScript® RT Reagent Kit with the gDNA Eraser from Takara Bio Inc. (Dalian, China) with 1 µg of the total RNA as template. The first-strand synthesis of cDNA for amplifying cDNA 3′ and 5′ ends was conducted with a SMARTer™ RACE cDNA Amplification Kit (Clontech, Palo Alto, CA, USA).

Full-length AlCPR cDNA amplification

Amplification of the conserved *CPR* segment was achieved by employing degenerate primers NADPHSP/NADPHASP (table 1). Furthermore, rapid amplification of cDNA end (RACE) was accomplished through a nested PCR approach using two combinations of gene-specific and generic primers (UPM and NUP applied by RACE cDNA Amplification Kit). The cloned *AlCPR* cDNA fragment was sequenced by Invitrogen (Shanghai, China). Subsequently, the complete sequence was validated through PCR utilizing targeted primers as listed in table 1.

Sequence and phylogenetic analysis

ProtParam tool (http://web.expasy.org/protparam/) was used to predict the AlCPR basic physicochemical parameters, such as

Table 1. Primers designed for cloning, RACE, qRT-PCR, RNAi and housekeeping gene.

Primer name	Primer sequences (5′-3′)	Primer function
NADPHFP	GARGAYGACTTYATYACDTG	Cloning
NADPHRP	TCDCCNGYNTCRTANCGCAT	Cloning
NADPH-3F	TCAACCAAAGACCGCCATTC	3′-RACE
NADPH-3FN	TCCGAGGAGAATGTAAGCAG	3′-RACE
NADPH-5R	CGTGAGATGTTCAGTAAGTT	5′-RACE
NADPH-5RN	TCCTGCTTACATTCTCCTCG	5′-RACE
AlCPRF	CATTCAATGAGCATGGAGGACG	Cloning full length
AlCPRR	CTAACTCCAAACGTCAGCGG	Cloning full length
q-AlNADPHF	ACACAGCGTTTTCCCGAGAC	qRT-PCR
q-AlNADPHR	CGCAAACGTAGATGTGCCCT	qRT-PCR
dsAlNADPHF	taatacgactcactatagggGGCCAACTGTTTGCGAGTAT	RNAi
dsAlNADPHR	taatacgactcactatagggGGTTCTGGGGTTAGACGTGA	RNAi
dsGFPF	ggatcctaatacgactcactatagggTGACCACCCTGACCTAC	RNAi
dsGFPR	ggatcctaatacgactcactatagggTTGATGCCGTTCTTCTGC	RNAi
RPL27F	GGGGTCACGTGAGTCATGGT	Housekeeping gene
RPL27R	GTCTGTTCGGTGACGAGGGT	Housekeeping gene

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molecular weight (MW) and isoelectric point (pI). Sequence identification and searching for orthologues were executed through the BLASTp tool (http://www.ncbi.nlm.nih.gov). The prediction of the catalytic residues and binding domains was conducted through the Conserved Domain Search service (http://www.ncbi.nlm.nih.gov/cdd/). ClustalW2 alignment tool available at the EBI website (http://www.ebi.ac.uk/Tools/msa/clustalw2/) was used for homology analysis of AlCPR and multiple other insect CPR homologues. The evolutionary relationships were delineated through a phylogenetic tree with MEGA 6.0 (Tamura *et al.*, 2013) using the neighbour-joining approach under bootstrap 1000 replicates.

Real-time quantitative PCR

The primers used for qPCR were listed in table 1. qPCR was proceeded with the ABI 7500 real-time PCR apparatus from Applied Biosystems, and the reaction mixture for each sample comprised 1 µl cDNA, 0.5 µl of each primer, 12.5 µl SYBR Green qPCR SuperMix-UDG and 10.5 µl of H_2O, adhering to the protocol outlined in the Invitrogen Platinum SYBR Green qPCR SuperMix-UDG kit. Reaction thermal and time conditions were as follows: UDG incubation at 50°C for 2 min, initial denaturation at 94°C for 2 min, succeeded by 40 repeated cycles of 94°C for 15 s and 60°C for 30 s. After the above procedure, the dissociation curve from 60 to 95°C was produced for testing the primer specificity. Three independent biological experiments were repeated. The $2^{-\Delta\Delta Ct}$ method as originally described by Livak and Schmittgen (2001) was applied for the calculation of gene relative expression levels. Reference gene *RPL27*, based on Luo *et al.* (2020) was used for normalisation in our study.

RNAi of AlCPR

The primers carrying T7 RNA polymerase promoter were shown in table 1 to amplify GFP and *AlCPR* fragments. Purified PCR amplicons served as the template of dsRNA synthesis with the MEGscript RNAi Kit (Ambion, USA). The quality and the concentration of the synthesised dsRNAs were confirmed through agarose gel electrophoresis and NAS-99 spectrophotometer (ACTGene, USA), respectively. The dsRNAs were dissolved in RNase-free water. The *A. lucorum* adults with 3-day-old were anaesthetised by exposure to carbon dioxide, and immediately put on ice for dsRNA injection. Approximately 1 µg ds*AlCPR* was administered via injection into the delicate area of the conjunctiva, specifically at the juncture of the prothorax and mesothorax in each *A. lucorum* adult with a Nanoliter 2000 Injector (WPI, USA). Negative and positive controls were treated with an equivalent RNase-free water and ds*GFPs*, respectively. Each treatment included three bio-repeats, with 15 adults per repeat. The *AlCPR* transcription levels were tested for determining RNAi efficiency after 24 h dsRNA injection.

Bioassay after RNAi

The pesticide technical lambda-cyhalothrin with 98% purity from Jiangsu Yangnong Chemical Co., Ltd (Yangzhou, Jiangsu, China) was used in our study and diluted in acetone. After 24 h dsRNA injection, a measured volume of the lambda-cyhalothrin preparation (0.6 µl $drop^{-1}$) microliters per drop was carefully applied to the thorax region of the mirid bugs employing a PB-600 semi-automatic dropper (PAT, USA). The doses of lambda-cyhalothrin applied on each adult were 9 and 30 ng $adult^{-1}$ (the dose value adopted LD_{50} of AL-S and LD_{20} of AL-R according to Zhen *et al.*, 2018), respectively. After 24 h lambda-cyhalothrin exposure, the mortality was tested. Three replications were conducted, and in each replication at least 20 adults were dropped by lambda-cyhalothrin solution.

Data analyses

Data statistical evaluation was performed with the GraphPad Prism 5 software. A two-tailed paired *t*-test, a type of Student's *t*-test, was implemented for determining the statistical significance of the difference between two groups. To assess the variability across multiple groups, a one-way ANOVA complemented by Tukey's post hoc test was conducted. Statistical significance was determined at the threshold of $P \leqslant 0.05$.

Results

AlCPR characterisation

The complete cDNA sequence of *AlCPR* with GenBank accession number: MG678043 possessed an ORF of 2052 bp nucleotides encoding 683 amino acid residues, while the lengths of 5′ UTR and 3′ UTR obtained were 272 and 234 bp nucleotides, respectively. In the 3′ UTR, a poly A was found without a typical polyadenylation signal site (AATAAA). The calculated *pI* and the estimated MW of AlCPR protein were identified as 5.19 and 76.82 kDa, respectively.

Signal peptide was absent within the deduced protein sequence of AlCPR. However, it was deduced that the AlCPR protein possesses an N-terminal membrane anchoring domain composed of 20 amino acid residues (20F–39L: FGPLDIVLLVALLGVALWWL) and was predicted at the AlCPR N-terminus, which was possibly involved in the orientation between AlCPR and the endoplasmic reticulum. All conserved function binding motifs of AlCPR, including the FMN-, FAD- and NADP-domains, were found in the primary structure of AlCPR (fig. 1). The detailed FAD combining sites in AlCPR was composed of a triplet of amino acids (Arg 460, Tyr 462 and Ser 463) (fig. 1A). Additionally, a pair of FMN connection sites were also discovered at the N-terminus of FMN domain. The catalytic site of AlCPR is composed of key residues including Ser 463, Cys 635, Asp 680 and Try 682 (fig. 1A). The amino acids forming the binding pockets of FMN, FAD and NADP in AlCPR were conserved with CPRs from other known insects by multiple alignments (fig. 1B). Alignment analysis showed that AlCPR shares 69–85% deduced amino acid identity with CPRs from other insects.

Phylogenetic relationship with CPRs from other insects

Phylogenetic analysis indicated that insect CPRs, when grouped in one branch together, originated from identical insect taxonomic orders, whereas Phthiraptera was observed to group within the Hemiptera branch. AlCPRs fell within the Hemiptera clade and most closed to *C. lectularius* CPR (ClCPR) (fig. 2), which was in accordance with the sequence alignment result. AlCPR and ClCPR from *C. lectularius* shared the highest identity with sequence comparison (table S1). Hence, AlCPR and ClCPR were firstly branched into the small group, and then clustered with other Hemiptera CPRs into Hemiptera branch.

Expression patterns of AlCPR across the various development phases and tissue

According to the quantitative reverse transcription PCR (qRT-PCR) results, *AlCPR* transcripts were found to be most

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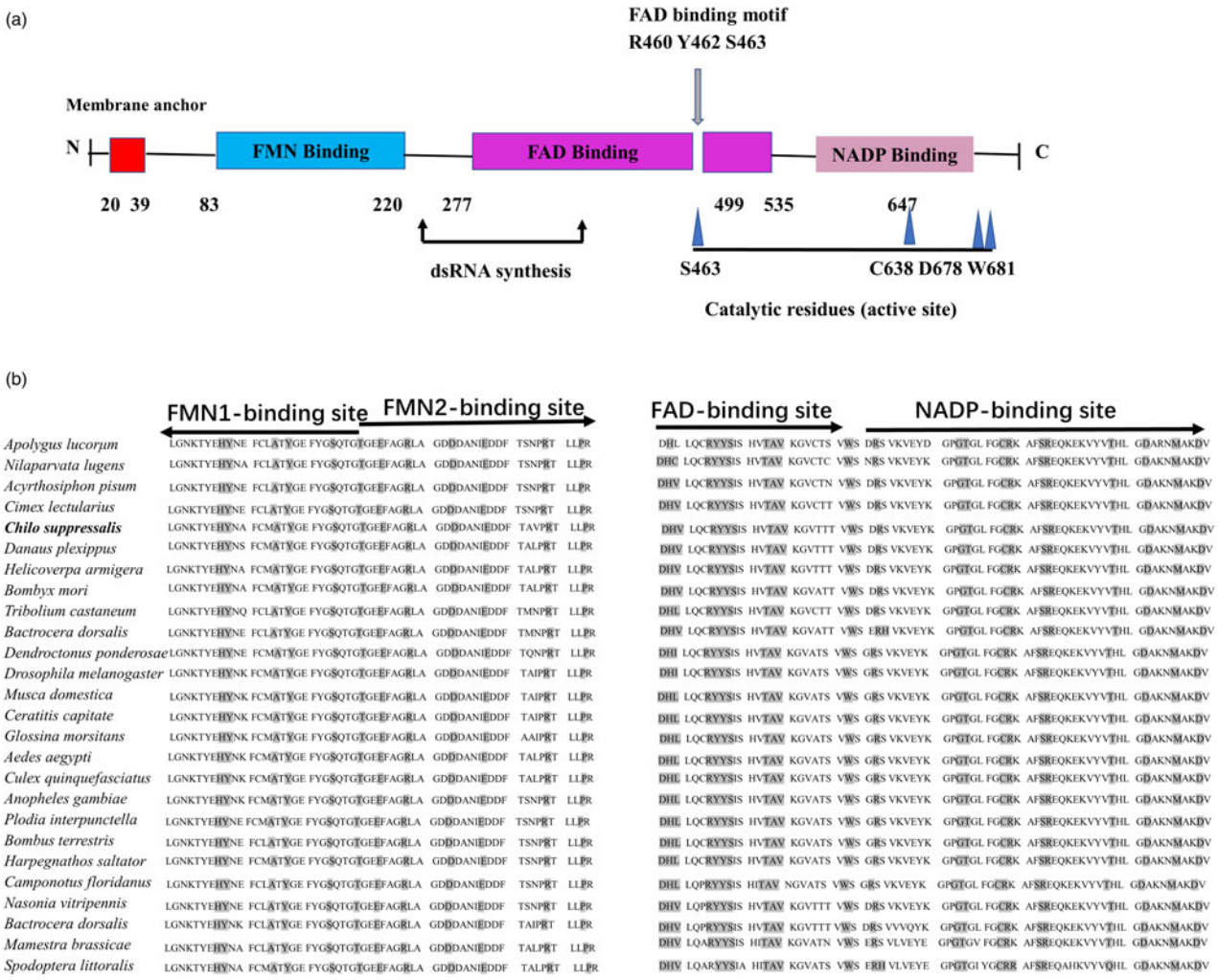

Figure 1. Structure of AlCPR. (A) Outline of AlCPR including membrane anchoring motif (red rectangle), conserved binding regions (FMN, blue rectangle; FAD, purple rectangle; NADP, pink rectangle), and catalytic residues. The segment allocated for dsRNA production is indicated. (B) Conserved domain alignment encompassing FMN, FAD and NADP. Binding site residues are shaded. The amino acid sequences of CPR were retrieved from NCBI.

abundant in the abdomen, whereas the lowest expression was detected in the head (fig. 3A). The transcription level of *AlCPR* was expressed during the whole development stages (fig. 3B). Furthermore, *AlCPR* expression remained relatively stable with no significant fluctuations during the 3rd–5th nymph stage (fig. 3B). The *AlCPR* mRNA expression levels in nymph stages 1st, 2nd, 3rd, 4th, 5th and adults were 3.35-, 4.23-, 5.87-, 6.13-, 5.48- and 13.29-fold of the transcript level in eggs (fig. 3B).

Expression profiles in LcR and SS strains

Compared to the SS strain, the LcR strain exhibited a pronounced increase (4.99-fold) in *AlCPR* transcript levels (fig. 4), suggesting *AlCPR* was most possibly involved in lambda-cyhalothrin resistance in *A. lucorum*.

Silencing AlCPR by RNAi

Following a 24 h exposure to ds*GFP*, the expression levels of *AlCPR* transcripts remained largely unchanged compared with DEPC-H_2O treated group (fig. 5). Ds*AlCPR* injection caused the *AlCPR* transcripts significantly decrease by 47.5 and 59.0% in the SS strain and the LcR strain, respectively (fig. 5).

AlCPR knockdown increases LcR strain susceptibility to lambda-cyhalothrin

After 24 h ds*AlCPR* treatment, the susceptibility to lambda-cyhalothrin was significantly elevated in the lambda-cyhalothrin-resistant LcR strain, with the mortality rate enhanced by 23.2% (fig. 6). Conversely, there was no significant increase in the sensitivity of the susceptible SS strain to lambda-cyhalothrin between ds*AlCPR* treatment and ds*GFP* control (fig. 6). These results revealed that silencing *AlCPR* only had a significant impact on the toxicity of lambda-cyhalothrin on resistant *A. lucorum*.

Discussion

In insects, the researches on the pyrethroids resistance mechanisms mainly focused on target site mutations, enhanced metabolic detoxification and epidermal thickening, especially in malaria vectors (Zhu *et al.*, 2020). Among the three major mechanisms

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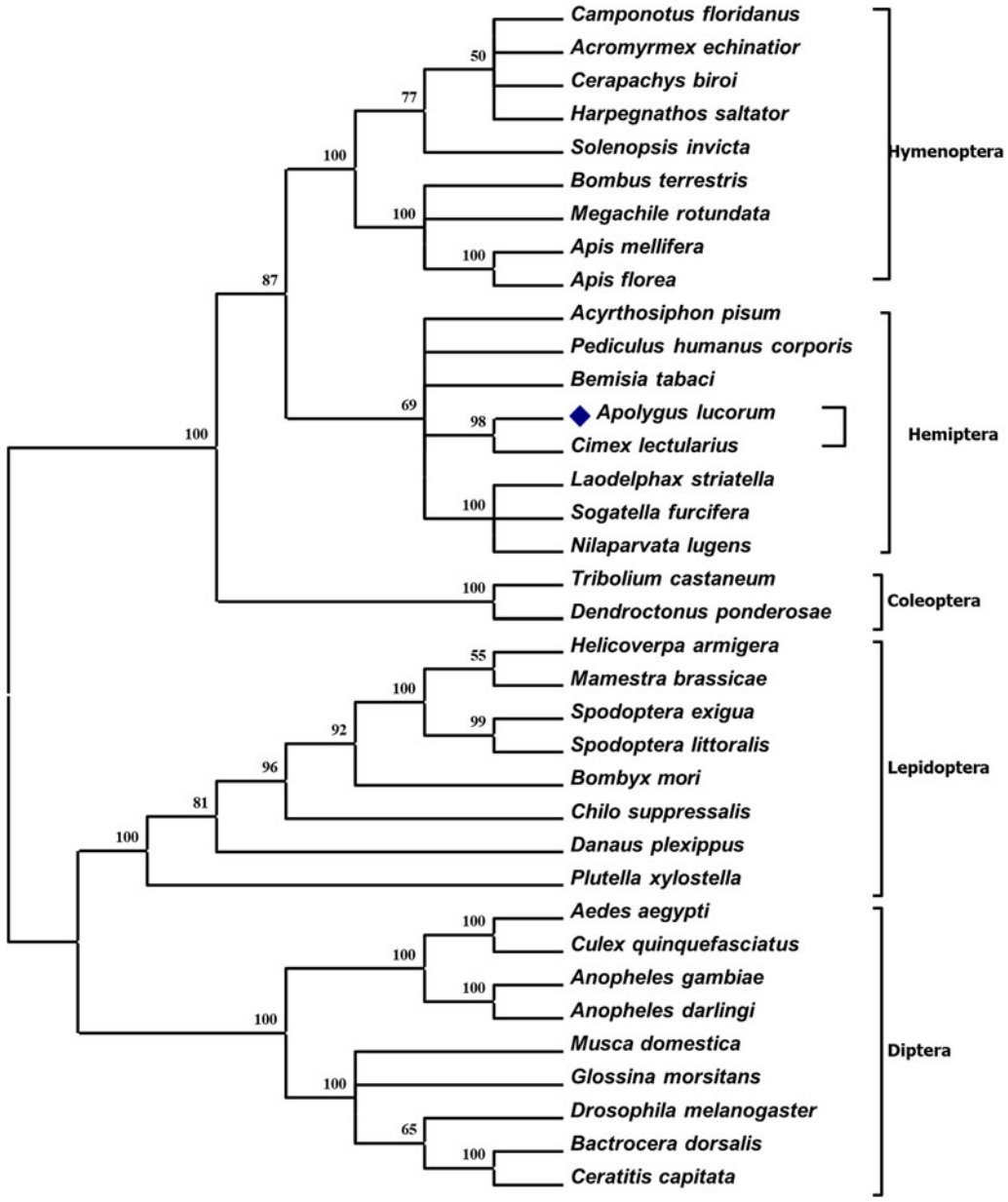

Figure 2. Phylogenetic relationship of AlCPR alongside other arthropod CPRs. The *A. lucorum* CPR is indicated by a dark blue rhombus. The GenBank numbers utilised to assemble the phylogenetic tree are detailed in table S1.

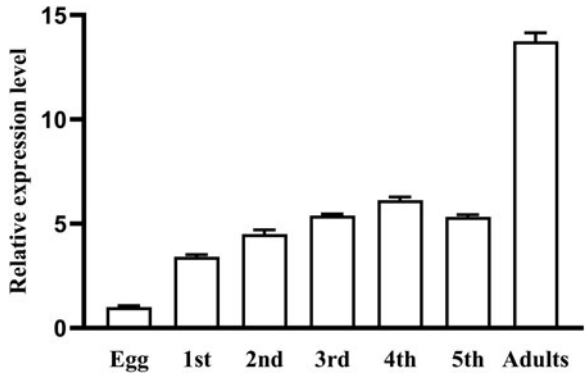

Figure 3. Expression profiles of *AlCPR* across various tissues in adults (A) and throughout the whole life stages (B).

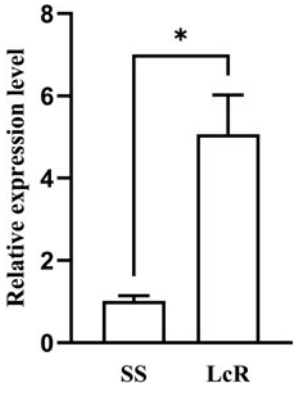

Figure 4. Expression level of *AlCPR* in SS and LcR strains.

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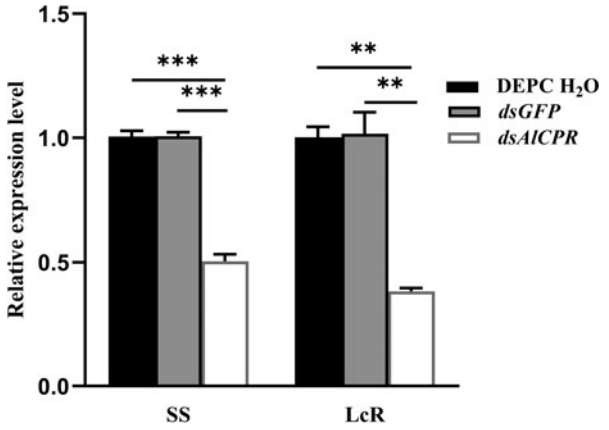

Figure 5. The *AlCPR* mRNA levels under *AlCPR* knockdown. SS, lambda-cyhalothrin-susceptible strain; LcR, lambda-cyhalothrin-resistant strain.

of pyrethroid resistance, target-site insensitivity (Soderlund and Knipple, 2003; Scott, 2019) and enhanced metabolic detoxification (Panini *et al.*, 2016) obtained more attention due to their importance (Liu, 2015; Smith *et al.*, 2016). Zhen and Gao (2016) pointed out that the *kdr* mutation (L1015F) was conferred for the lambda-cyhalothrin resistance in one *A. lucorum* field population collected from Shandong, Binzhou transgenic *Bt* cotton. Meanwhile, the metabolic mechanism mediated by overexpressed CYPs was also identified in *A. lucorum* lambda-cyhalothrin-resistant strain (Zhen *et al.*, 2018).

CYPs are verified to participate in the insecticide resistance development across numerous insect species (Liu *et al.*, 2015b; Li *et al.*, 2021; Nauen *et al.*, 2022). In the P450 system, CPR served as an indispensable electron donor necessary in transferring the electron(s) from NADPH to CYPs, then CYPs catalyse the metabolism of various endogenous substances and xenobiotic chemicals (Pandey *et al.*, 2007). Further research on identification and characterisation of insect CPR would be helpful to reveal the detoxification of xenobiotics, especially insecticides mediated by cytochrome P450s via CYPs/CPR system.

In our research, *AlCPR*, a NADPH-CPR, was obtained from *A. lucorum*. The expression patterns and potential role in insecticide resistance were characterised. Bioinformatics analysis revealed that a membrane anchor at N-terminus, three types of the

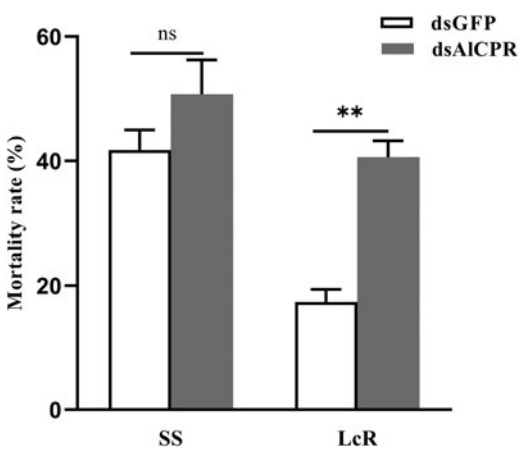

Figure 6. Mortality of *A. lucorum* adults to lambda-cyhalothrin in the SS strain (A) and LcR strain (B) after silencing *AlCPR*.

conserved binding domains, including FMN, FAD and NADP binding regions, among these motifs, the critical catalytic residues were overall confirmed (fig. 1). The membrane anchor at AlCPR N-terminus is hydrophobic, facilitating the protein molecule anchoring to the endoplasmic membrane in the P450 catalytic cycle and involving the electron transfer via a correct spatial interaction between the CPR protein and CYPs (Wang *et al.*, 1997; Laursen *et al.*, 2011). The FMN and FAD/NADPH binding domains were situated at the N-terminus and near the C-terminus, respectively. These two domains interconnected each other with random coils. Two FMN binding domains were identified in AlCPR including FMN1 and FMN2, suggesting their crucial function on the CYP interaction. Meanwhile, three highly conserved amino acids (R460, Y462 and S463) were found in the FAD binding region of AlCPR. Four conserved amino acid residues (S463, C635, D680 and W682) consisted of AlCPR catalytic centres, which was essential for the hydride transfer circulation. Therefore, the fundamental architecture of CPRs exhibited remarkable conservation across diverse biological groups, which demonstrated the vital role of this enzyme in evolutionary progression (Laursen *et al.*, 2011).

The *AlCPR* transcript expression pattern was determined throughout the whole life-cycle phases and across a range of tissues. For stage-expression profile, *AlCPR* transcripts were detected at every developmental stage, with the most abundant at adult stage (fig. 3B), suggesting that *AlCPR* might be important in the development of *A. lucorum* and have a crucial role in adults. This expression trend similarly was found in *C. lectularius ClCPR* (Zhu *et al.*, 2012), *T. cinnabarinus* (Shi *et al.*, 2015) and *L. migratoria* (Zhang *et al.*, 2017). However, the *CPR* displayed variable expression profiles during insect development stages among species. Generally, protein tissue distribution was usually associated with its role in certain tissues. In insect abdomen part, there were many important tissues that existed in this section, such as metabolism detoxification organs, including midgut, fat body and Malpighian tubule. It is worth noting that the highest *AlCPR* mRNA level was found in abdomen. This tissue distribution profile was similar to *C. lectularius CPR* (Zhu *et al.*, 2012) and *N. lugens CPR* (Liu *et al.*, 2015a), all potentially indicating CPR may be involved in the xenobiotic metabolism by P450s.

Multiple researches have verified that up-regulation of *CPR*s in insect is involved in insecticide resistance. The significant enhancement of *CPR* mRNA level (3.12-fold) was discovered in a fenpropathrin-resistant *T. cinnabarinus* strain in contrast to a susceptible strain (Shi *et al.*, 2015). The significantly increased mRNA expression level of *HaCPR* was found in *H. armigera* midgut from fenvalerate-resistant strain, contrasting with the susceptible strain (Tang *et al.*, 2012). In the rice leaffolder, *Cnaphalocrocis medinalis*, exposed to non-lethal concentrations of pesticides such as abamectin, chlorantraniliprole and chlorpyrifos, the expression of *CmCPR* was up-regulated (Zhang *et al.*, 2018). In the *β*-cypermethrin-resistant strain of *Plutella xylostella*, the *CPR* mRNA expression was significantly enhanced with 12-fold increase in contrast to the *β*-cypermethrin-susceptible strain (Chen and Zhang, 2015). Knockdown of CPR increased the susceptibility against permethrin in the mosquito *An. gambiae* (Lycett *et al.*, 2006). *ClCPR* knockdown only strikingly increased the susceptibility of resistant bed bug population against deltamethrin, but not in the susceptible *C. lectularius* (Zhu *et al.*, 2012). Silencing the *NlCPR* gene markedly enhanced the susceptibility of *N. lugens* nymphs against imidacloprid and *β*-cypermethrin (Liu *et al.*, 2015a). The fenvalerate susceptibility of *H. armigera* was remarkably increased with ds*CYP6B7* and ds*CPR*

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mixture treatment in the HDFR strain (He *et al*., 2020). In *S. litura*, the suppression of *SlCPR* via RNAi led to enhanced phoxim susceptibility (Ji *et al*., 2019). Similarly, in the two-spotted spider mite, *T. urticae*, down-regulation of *TuCPR* reduced the resistance to three acaricides, fenpyroximate, bifenthrin and abamectin (Adesanya *et al*., 2020). In *Sitophilus zeamais*, knockdowning *SzCPR* resulted in the increased sensitivity to terpinen-4-ol (Huang *et al*., 2020). The thiamethoxam and imidacloprid susceptibility was significantly increased when *DcCPR* silencing in Asian citrus psyllid *Diaphorina citri* (Yuan *et al*., 2021). In *S. litura*, knocking down *SlCPR* significantly increased the susceptibility against indoxacarb in both SS and InRS strains (Shi *et al*., 2021). In the pea aphid *Ac. pisum*, silencing *ApCPR* caused a significantly increased susceptibility to three contact insecticides, encompassing chlorpyrifos, imidacloprid and betacypermethrin (Qiao *et al*., 2022). Tang *et al*. (2023) found that the susceptibility of *Ap. gossypii* against sulfoxaflor increased during silencing of *AgCPR*. Our findings indicated that the *AlCPR* expression was notably elevated in the lambda-cyhalothrin-resistant LcR strain, contrasting with the levels observed in the SS strain (fig. 4), suggesting P450 system was possibly associated with the lambda-cyhalothrin resistance development, consistent with the previous conclusion that the overexpression of numerous cytochrome P450 genes was observed in the LcR strain of *A. lucorum* (Zhen *et al*., 2018). In LcR strain, no *kdr* mutation was found, implying the detoxification metabolism playing main role in the lambda-cyhalothrin-resistant development. Besides, suppression of *AlCPR* by ds*CPR* injection could significantly enhance the susceptibility to lambda-cyhalothrin in LcR strain (fig. 6). All these results implied a significant function for CPR in the CYPs-mediated metabolism resistance against lambda-cyhalothrin in LcR strain.

In conclusion, the transcript profile of *AlCPR* was characterised from *A. lucorum*. Furthermore, knockdowning *AlCPR* only enhanced the susceptibility of *A. lucorum* to lambda-cyhalothrin in the lambda-cyhalothrin-resistant strain (LcR). All these findings indicated that *AlCPR* was probably involved in the P450-mediated detoxification of lambda-cyhalothrin. In future, the CPR activity assay between SS and LcR strain should be conducted by assessing and comparing the activity of NADPH-dependent cytochrome C reductase. Besides, the heterologous over-expression of *AlCPR* in Sf9 cells and cytotoxicity assays with lambda-cyhalothrin at different concentrations were executed to uncover the real role of *AlCPR* in the resistance of *A. lucorum* to lambda-cyhalothrin.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0007485324000488

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