Identification of cuticular protein genes in *Glyphodes pyloalis* (Walker) and expression patterns under stress of insecticides

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Glyphodes pyloalis Walker (Lepidoptera: Pyralidae) is one of the major destructive agricultural pests, which is widely distributed in the main mulberry planting areas in eastern China and other countries (Shao et al., 2020). This pest has been suppressed excessively dependent on commonly used insecticides, such as phoxim, cypermethrin and chlorfenapyr. Insect cuticle is composed mainly of structural proteins and the polysaccharide chitin. Depending on the location and type of cuticle, cuticular proteins have effects on shaping insect bodies and influencing insect development stages. (Soares M. et al., 2007). Insect cuticular proteins can be divided into 12 families according to chitin-binding domain (ChtBD) (Willis JH et al., 2010). The CPR family is the largest family of cuticle proteins (CPs), which can be further divided into two subgroups based on the presence of one of the presumptive chitin-binding sequence motifs denoted as Rebers-Riddiford (R&R) consensus sequence motifs RR-1, RR-2, RR-3 (Tribolium castaneum RR-1 Cuticular Protein TcCPR4 Is Required for Formation of Pore Canals in Rigid Cuticle). However, the exact roles of CPRs in insecticides tolerance in *Glyphodes pyloalis* still receive less attention.

In the present study, we identified several *GpCPRR* genes from the transcriptome database of *G. pyloalis*. Two types of GpCPR protein have been identified, which can be divided into two subgroup based on Rebers-Riddiford (R&R) consensus sequence motifs: RR-1, and RR-2. Four of GpCPRR proteins contains the RR-1 cuticular protein motif of G-X(8)-G-X(6)-Y-X-A-X(4)-G. Three GpCPRR-2 proteins with the motif of G-X-Y-X(6)-G-X(4)-V-X-Y-X(2)-D-X(3)-G were identified(Figure 1). All *GpCPRR* genes contained full-length open reading frames (ORF) and were relatively high similar with CPR proteins of other insects like *Ostrinia furnacalis*(Table 1). Furthermore, we measured the expression levels of three *GpCPRR* genes after exposing to chlorfenapyr, lambda-cyhalothrin, Phoxim and deltamethrin by qRT-PCR (Figure 2). The results showed that the expression of *GpCPRR1.8* was significantly induced in *G. pyloalis* by chlorfenapyr, the main pesticide used against this pest. Compared with the control, The expression of *GpCPRR1.11* was significantly up-regulated after 48h exposure to two contact toxicity insecticides lambda-cyhalothrin and deltamethrin. Four insecticides all have significant effects on the expression of *GpCPRR2.15* and the upregulation level depended on exposure time. The expression of three *GpCPR* genes decreased continuously with increasing exposure time. The results provide a basis for further functional investigation of CPR implied in insecticide stress in *G. pyloalis*.

Table 1 Sequence information of identified *GpCPRR* genes

Clan	Gene Name	ORF (aa)	Blastp Results	E value
GpCPRR-1	*GpCPRR1.8*	310	Larval cuticle protein LCP-30-like [*Ostrinia furnacalis*]	2.4E-36
	GpCPRR1.11	128	Larval cuticle protein LCP-14-like [*Ostrinia furnacalis*]	3E-28
	GpCPRR1.14	105	Flexible cuticle protein 12-like [*Ostrinia furnacalis*]	2E-35
	GpCPRR1.23	73	Larval cuticle protein 1-like [*Galleria mellonella*]	5.4E-23

	GpCPRR2.11	183	Cuticle protein 7 [*Ostrinia furnacalis*]	4.7E-64
GpCPRR-2	*GpCPRR2.15*	177	Cuticle protein 7-like [*Ostrinia furnacalis*]	6E-52
	GpCPRR2.17	171	Cuticular protein RR-2 motif 130 [*Antheraea pernyi*]	4.7E-84

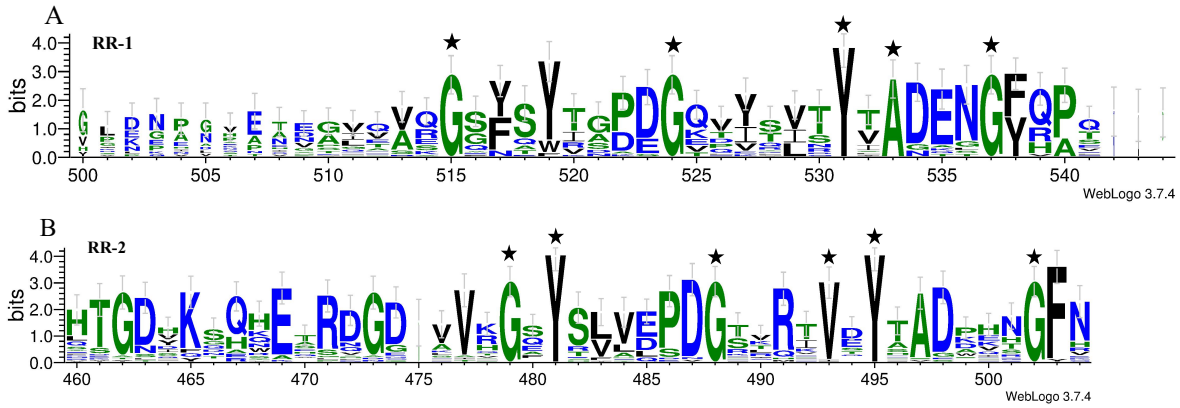

Figure 1 Conserved motif analysis of RR-subfamily proteins of *Glyphodes pyloalis* (Walker)
A: RR-1 subfamily B: RR-1 subfamily

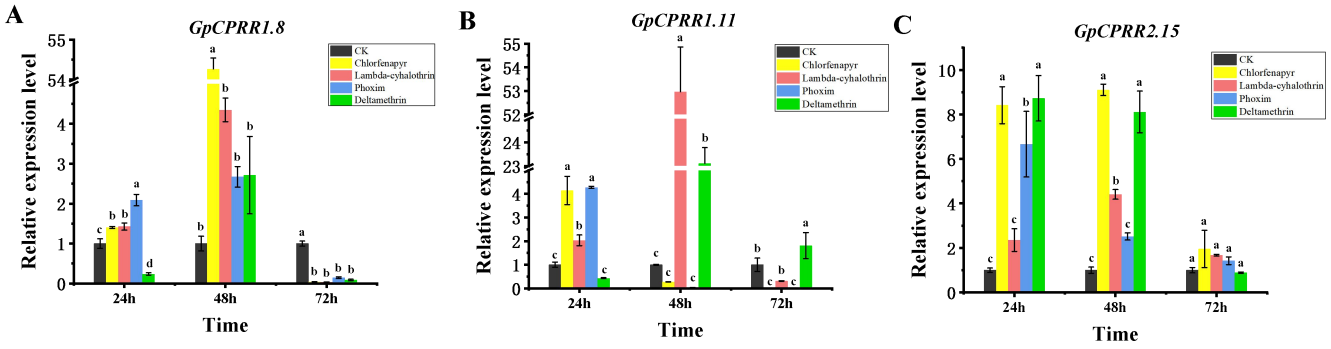

Figure 2 Relative expression level of *GpCPRR1.8*(A), *GpCPRR1.11*(B) and *GpCPRR2.15*(C) after exposing to chlorfenapyr, lambda-cyhalothrin, phoxim and deltamethrin

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