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Transcriptional Changes in nAChRs, Interactive Proteins and P450s in *Locusta migratoria manilensis* (Orthoptera: Acrididae) CNS in Response to High and Low Oral Doses of Imidacloprid

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ABSTRACT. The insect central nervous system (CNS) is the target for many insecticides, and changes in transcript levels could be expected after insecticide applications. In this study, differentially expressed genes in the locust (*Locusta migratoria manilensis*) CNS in response to imidacloprid treatments at low dose (LD, 10% mortality) and high dose (HD, 80% mortality) were identified. Two nicotine acetylcholine receptor (nAChR) subunits genes and 18 interacting protein genes were regulated at LD, and only one nAChR subunit gene and 11 interacting proteins were regulated at HD. Among the 110 annotated P450 unigenes, 43 unigenes were regulated at LD and 34 unigenes were regulated at HD. Most of the differentially expressed P450 unigenes were mapped to CYP4, in which most unigenes were upregulated at LD, but downregulated at HD. Totally, the numbers and regulation levels of the regulated genes were more at LD than that at HD. Seventeen unigenes were selected to test their expression changes following insecticide treatments by qRT-PCR, in which the changes in more than half of the selected genes were verified. The results revealed the variation in the response of locusts to different insecticide pressure, such as different doses.

Key Words: *Locusta migratoria manilensis*, imidacloprid, nAChR, interacting protein, P450

In insects, nicotinic acetylcholine receptors (nAChRs) are neurotransmitter-gated ion channels that mediate fast cholinergic synaptic transmission (Sattelle 1980, Matsuda et al. 2001). As the main neurotransmitter receptors, nAChRs are ideal targets for several kinds of insecticides, such as neonicotinoids. Several proteins, such as chaperones, regulators, and modulators, play important roles in the synthesis, degradation, and functional regulation of insect nAChRs, in vivo and in vitro (Lansdell et al. 2005, Choo et al. 2008, Millar 2008). RIC-3 and Lynx are two noted regulators of nAChRs in insects (Choo et al. 2008, Millar 2008, Liu et al. 2009, Yang et al. 2010). RIC-3 is necessary for proper folding/assembly of nAChRs, functioning as a molecular chaperone (Millar 2008). Lynx proteins improved the efficiency of protein folding and assembly in *Pyrocoelia rufa* and *Nilaparvata lugens* (Choo et al. 2008, Liu et al. 2009, Yang et al. 2010). Recent studies showed that nAChR subunit expression was regulated by imidacloprid, which changed the sensitivity to neonicotinoid insecticides (Markussen and Kristensen 2010, Yu et al. 2011, Taillebois et al. 2014). Thus, insect nAChRs and their interacting proteins might be regulated by insecticides during transcription.

The important proteins in insect central nervous system (CNS) are targets for many insecticides and insects can use the CNS as the last line of defense against insecticides. In insects, P450 monooxygenases are the most important detoxification enzymes to metabolize endogenous and exogenous compounds such as insecticides. In vertebrate CNS, P450s in the brain could catalyze neurosteroids and drugs (Strobel et al. 2001, Miksys and Tyndale 2009). Some P450 enzymes in insects, such as *Drosophila melanogaster* CYP6G1, *Bemisia tabaci* CYP6CM1vQ, and *Nilaparvata lugens* CYP6AY1 could metabolize imidacloprid (Joussen et al. 2008, Karunker et al. 2009, Ding et al. 2013). The induction/repression mechanisms of detoxification enzymes, in response to xenobiotic exposure, have been elucidated in some insect species (Feyereisen 1999). Expression of CYP4 and CYP9 genes could be induced by compounds present in the midgut of *Manduca sexta* (Snyder et al. 1995, Stevens et al. 2000). However, there are few reports regarding the induction of P450s in insect CNS by insecticides. Therefore, the variation in P450 expression in insect CNS in response to imidacloprid exposure merits an investigation.

The locust [*Locusta migratoria manilensis* (Meyen)] is an ideal model hemimetabolous insect and causes tremendous loss of agricultural production (Hassanali et al. 2005). In this study, we used the Illumina sequencing platform to analyze genes differentially expressed in the locust CNS following application of various doses of imidacloprid. The putative interacting proteins of nAChRs and P450s showed marked variation in their expressions. The expression variations of some unigenes were validated by qRT-PCR. This study provides useful information regarding gene regulation in insect CNS in response to insecticide application.

Materials and Methods

Insects and Chemicals. Locusts were purchased from the Hongguang Medicinal Animal Co. Ltd. (Jurong, Jiangsu, China).

Imidacloprid (97%) was purchased from Red Sun Group Corporation (Nanjing, China).

Bioassays and Sample Collection. Bioassays were performed in triplicate using 30 adult locusts with mixed sexes. Each locust was fed with 10 μL of insecticide solution with pipettes. The locusts that could not take in the entire insecticide dose were eliminated from the study. Imidacloprid was first dissolved in acetone and then diluted into different imidacloprid solutions using distilled water with the final proportion for acetone and Triton X-100 of 0.1 and 0.5% by volume. Five imidacloprid concentrations from 10 to 160 mg/liter were used. Water with acetone and Triton X-100 at 0.1 and 0.5% by volume was applied as the control (CK). All tests were conducted at 25 ± 1°C and mortalities were recorded after 24 h. The data were analyzed using DPS software (Tang and Zhang 2013).

Table 1. The specific primers used in qRT-PCR

Unigene ID	Putative annotation	Primers
CL2087-1	CYP6AM1	F—ATCGACTACACTTACTGGAA
		R—GAGTAAATACCGACGAAATC
U5019	CYP4G43	F—GCCTTCTTGGACCTTATG
		R—GTCCTGAATGTCCTGATG
CL2702-3	CYP6BK17	F—CTCGACAAGGTCGTCTCAGAGACG
		R—GAAGTAGTGAGGGTCGTGATGGAG
U33624	Protein kinase C	F—CCTGAATGGTGGAGATCTGATG
		R—GCGGATATGGCCTTCATAGTC
CL2319-1	Mito 12A2	F—GTTCGCCGAGCTAGAGGTCTAC
		R—GTCTCTGTCGATGACCTTGAAC
U13389	CYP4C1	F—CAAACGTGAAGATCGATTGCGC
		R—GGGTGAAGGAAGGCATACATAC
CL2611-1	CYP4C1	F—CAGGATCAGTGAACTCACCATG
		R—CTTGGATTGGGCTCCGTTCG
U21296	Spectrin alpha chain	F—GAACCTGTTGAGCGTGTTGAGGAG
		R—CATCCTCCCTTCGAACATTCCACC
U29431	Spectrin alpha chain	F—ATCTATGATGGAAGGTTCTG
		R—CTGTGCTCTGTATACCTATT
CL4026-1	CYP6AM1	F—AAAGCTATTTCACTCTGGAG
		R—TTCAGTACCTCGTCTATCTC
U6027	CYP4C1	F—CACCTACGTCATGCTCTACA
		R—AGTAATGAGGTCGCTGCTAC
CL2853-2	CYP4C62	F—CCGATGTTCAGGAGAAGGCATAC
		R—CGTAAGCGTACGGCCTATGAAAG
U31578	Mito 2U1	F—CTGATATACACGGCGCACCACC
		R—GACAGAGATGGGAGTGAGAGTCC
U31293	Mito 302A1	F—GCAGGGACTGTGGTTGTCACACAG
		R—GAGCGATACAGCTGCGTGGTCCATG
CL181-1	Dynamin 1	F—ACTAAGGTTCCTGTGGGAGATC
		R—CATCTGGATCAACACTCTTGGC
U38505	CYP4D2	F—CTGCACTTCTGGCGCAAGATGC
		R—GTCACGTACTCGTCGCGCTC
CL3168-1	Nlα8	F—CAACTACGAGGTGACGCTGATG
		R—CCGTTGTAAGTCCACGATCCAA
β-actin	β-actin	F—CGAAGCACAGTCAAAGAGAGGTA
		R—GCTTCAGTCAAGAGAACAGGATG
GAPDH	GAPDH	F—GATGTGAAAGCCGAAGGAAACTG
		R—GTTGGAGATGACCTTGTAAGAGG

Table 2. The expression of nAChR subunits after exposure to imidacloprid

Name	Unigene ID	Log_2Ratio (CK-LD)	Log_2Ratio (CK-HD)
α1	CL4363-2	−0.93	0.02
α2	U7233	−0.39	0.06
α4	CL3101-1	−1.75	−0.41
	CL4363-1	−1.10	−0.33
α5	U4231	0.52	−0.30
α6	U17972	0.27	0.24
α7	CL2382-2	0.42	0.57
α8	CL3168-1	0.50	1.20
	U21252	−1.26	0.02
	U8705	−8.44	−2.57
α9	U4117	0.33	0.19
β1	U23042	−0.66	−0.42

The low (LD_{10}), high (LD_{80}) doses of imidacloprid, and the control (CK) were applied to adult locusts. After 24 h, the entire CNS from five surviving locusts as one sample was isolated by dissection for subsequent RNA isolation.

RNA Isolation, Library Construction, and Illumina Sequencing. The entire CNS was homogenized and RNA was extracted utilizing the TRIzol reagent (Invitrogen, USA), following the manufacturer's instructions. RNase-free DNase I (Takara Biotechnology, China) was applied to eliminate genomic DNA contamination.

cDNA library preparation and sequencing reactions were performed by the BGI (Shenzhen, China). The library was sequenced using Illumina HiSeq 2000 (Illumina Inc., San Diego, CA).

Analysis of Illumina Sequencing Results. Reads from each library were assembled separately after elimination of low-quality reads. Trinity software was used for de novo transcriptome assembly (Grabherr et al. 2011) to generate contigs and unigenes. In the final step, the transcripts were searched for similarity against the protein databases NR, Swiss-Prot, KEGG, and COG, using BLASTX with a cut-off *E*-value of 10^{-5}. The best-aligned results were used to determine the direction of sequences. These unigenes were initially aligned using protein databases, in the priority order NR, Swiss-Prot, KEGG, and COG. If a unigene could not be aligned to any database, its sequence direction and predicted coding region were determined using the ESTcan software (Iseli et al. 1999).

Differential Expression of Unigenes. Unigene expression was determined using the fragment per kilobase of transcript per million fragments (FPKM) method. The FPKM and reads per kilobase per million read (RPKM) formulae are identical (Mortazavi et al. 2008). However, when both pairs of reads were aligned to a gene, they were treated as one fragment with FPKM, but two reads by RPKM. An algorithm was developed to identify genes differentially expressed between two samples (Audic and Claverie 1997). False discovery rate (FDR) control is a statistical method used in multiple hypothesis testing. Sequences according to 'FDR ≤ 0.001 and the absolute value of $\log_2\text{Ratio} \geq 1$' were regarded as being significantly differentially expressed.

qRT-PCR Validation of Gene Expression. Seventeen unigenes were selected to test their expression in the locust CNS following imidacloprid treatments at low and high doses. The total RNA was extracted from the locust CNS, and at least three replicate experiments were prepared for each sample. Reverse transcription to cDNA was performed using the PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa, Tokyo, Japan) and qRT-PCR was performed with the SYBR *Premix Ex Taq* (TaKaRa) on a 7500 Real-Time PCR System (Applied Biosystems) with 10 μL reaction buffer (SYBR *Premix Ex Taq*), 0.4 μL each primer (10 μM), 0.4 × Rox Reference Dye II, H_2O up to 20 μL, following the manufacturer instructions. Gene expression difference was calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001). For normalization, two reference genes (β-actin and GAPDH) were validated experimentally for each generation and treatment, with the geometric mean of the selected genes then used for normalization according to the strategy described previously (Vandesompele et al. 2002). All PCR primer sequences are shown in Table 1.

Results and Discussion

Bioassays. Feeding bioassays were used to assess imidacloprid toxicity in locusts. The toxicological regression line (LD-P) was computed as $y = 1.0398x + 5.2032$. The LD_{50} of imidacloprid for the locust was 0.64 mg (95% CI 0.51–0.80), with control mortality of 4.44%. According to the LD-P line, LD_{10} (0.037 mg) and LD_{80} (4.11 mg) were selected as the low-dose (LD) and high-dose (HD) to treated locusts, respectively.

Expression of nAChR Subunits. To gain insight into the molecular biology of the response to insecticide stress, transcript levels of the target genes, nAChR subunits, were analyzed. A total of 22 nAChR subunit-encoding sequences, originated from CK, LD, and HD reads, were identified in the locust CNS transcriptome. An algorithm (see 'Materials and Methods' section) was used to identify genes differentially expressed at LD and HD. Partial data are shown in Table 2 and detailed data in Supp Table 1 (online only). The unigenes annotated as Locα4 and Locα8 showed a significant decrease in expression at LD, and Locα8 expression was also significantly regulated by HD imidacloprid (Table 2). At HD, there are three unigenes annotated as α8 subunit, in which one unigene was upregulated and another one was downregulated. Therefore, the expression variation of Locα8 required further verification.

Recent studies have shown that changes in nAChR subunit expression caused decreased sensitivity or resistance to neonicotinoids (Markussen and Kristensen 2010, Yu et al. 2011, Taillebois et al. 2014).

We suggest that the variation in nAChR subunit expression induced by imidacloprid treatment might contribute to the decreased sensitivity to imidacloprid. In previous studies of neonicotinoid resistance, the selection of a resistant strain or population was indispensable, which always was time-consuming. By contrast, the findings on changes in the subunit expression following imidacloprid treatments may allow rapid identification of insensitivity due to changes in expression levels of nAChR subunits.

nAChRs-Interacting Proteins. The intracellular factors involved in the regulation of nAChR function could modulate neonicotinoid sensitivities (Bodereau-Dubois et al. 2012). The cAMP-dependent protein kinase and protein phosphatase 1/2A are known to interact with nAChRs and regulate the functions of nAChRs through their involvement in the phosphorylation or dephosphorylation process. These protein kinases have been shown to reduce the potency of imidacloprid on insect nAChRs (Courjaret and Lapied 2001). Therefore, it is appropriate to determine whether insecticides regulate the function of nAChRs through the modulation of expression of these regulator and modulator proteins.

For nAChRs interacting proteins, 18 and 11 unigenes were regulated at LD and HD, respectively. Partial data are provided in Table 3 and detailed data in Supp Table 2 (online only). A total of 15 genes (14 upregulated and one downregulated genes), encoding interacting proteins, were expressed significantly differently at LD. All of the 11 significantly differentially expressed genes at HD were upregulated. Some proteins interacted with nAChRs and potentially regulated the expression of insect nAChRs. These genes, encoding RIC-3, SAP102, ubiquilin-1, PICK1, *N*-ethylmaleimide-sensitive factor, pyruvate kinase 3, spectrin alpha chain and protein kinase C, were upregulated at both LD and HD (Table 2). The $\log_2$Ratio values of these genes at LD were higher than those at HD. Several genes, such as VAMP-1/2 and dynamin 1, were specifically regulated at LD. There were also some genes specifically regulated at HD, such as the lysozyme C-1 precursor and 14–3–3 protein. These results suggested that the expression of nAChR-interacting proteins was differently regulated by imidacloprid at different doses. These proteins might regulate the function of nAChRs through changes in their expression levels in response to insecticide-induced stress. Of course, these factors merit further investigation.

Dynamin1 (CL181-1) was downregulated at LD, but was unaffected at HD. The different regulation patterns for this gene by an applied dose could be regarded as a signal to define that the dose was a lethal dose or a sub-lethal dose. Upon application of a fixed insecticide dose, insects might be regarded relatively susceptible if the regulation pattern is consistent with that of HD and relatively resistant if consistent with LD. So, after the confirmation in different populations, these differently regulated genes could be developed into molecular markers to estimate the resistance levels in insects.

Expression of P450-Encoding Genes. The expression levels of 54 P450-enoding genes in the locust CNS transcriptome were found to vary (Supp Table 3 [online only]). A total of 43 P450 unigenes, including 32 upregulated unigenes and 11 downregulated unigenes, was differentially expressed at LD. Thirty-four P450 unigenes, including 21 upregulated and 13 downregulated unigenes, were differentially expressed at HD. For both LD and HD, the CYP4 subfamily had the greatest number of differentially expressed genes of all P450 subfamilies. Fourteen upregulated and three downregulated unigenes at LD belonged to the CYP4 subfamily, and the number was 16 at HD, including seven upregulated and nine downregulated unigenes (Table 4). This may be because the CNS P450s are primarily involved in producing hormones rather than metabolize xenobiotics (Ishizaki and Suzuki 1994, Bogus and Scheller 1996). For example, the CYP4 gene family in insect CNS is involved in regulating the production of molting hormone and juvenile hormone (Sutherland et al. 1998, 2000, Maibeche-Coisne et al. 2000, Aragon et al. 2002, Niwa et al. 2011). It is reasonable to assume that a greater number of genes in the CYP4 subfamily, compared with the CYP6 subfamily, were regulated by the insecticide imidacloprid in the locust CNS. P450 CYP4G15 is expressed specifically in the CNS of *D. melanogaster* (Maibeche-Coisne et al. 2000). Some unigenes were annotated as CYP4G15 in the locust CNS and regulated by imidacloprid treatment.

In insects, the CYP6 P450 subfamily plays important roles in metabolizing imidacloprid (Ding et al. 2013, Riaz et al. 2013, Yang et al. 2013).

Table 4. Numbers of genes encoding P450 proteins whose expression was regulated by imidacloprid treatments

	LD		HD	
	Upregulated	Downregulated	Upregulated	Downregulated
CYP4	14	3	7	9
CYP6	9	4	7	2
CYP9	4	2	3	0
Mito	5	2	4	2
Total	32	11	21	13

Table 3. The expression of some nAChR-associated proteins after exposure to imidacloprid

Name	Unigene ID	$\log_2$Ratio (CK-LD)	$\log_2$Ratio (CK-HD)	Function
RIC-3	U31006	1.15	1.02	nAChR maturation (Millar 2008)
SAP102	CL2460-1	1.65	0.77	Anchor postsynaptic proteins (Conroy et al. 2003)
	CL2460-4	0.94	1.02	
ubiquilin-1	CL1030-2	3.02	1.83	nAChR subunit degradation (Ficklin et al. 2005)
PICK1	CL1823-1	1.34	1.10	Reduce nAChR clusters (Baer et al. 2007)
VAMP-1/2	U16086	1.46	0.48	Synaptic vesicle protein (Farias et al. 2007)
COP9 signalosome complex subunit 6	U10234	1.22	0.52	interacting with human β1 (Stelzl et al. 2005)
Clathrin heavy chain	CL490-1	1.92	0.93	nAChR trafficki (Kabbani et al. 2007)
N-ethylmaleimide-sensitive factor	U545	1.86	1.20	nAChR trafficking (Kabbani et al. 2007)
eEF2	U17330	1.61	0.70	nAChR maturation (Kabbani et al. 2007)
G-protein (Goα)	CL4547-1	1.31	0.69	interacting with mouse β2 (Kabbani et al. 2007)
Pyruvate kinase 3	CL4699-3	1.53	1.26	interacting with mouse β2 (Kabbani et al. 2007)
Spectrin alpha chain	U21296	2.37	2.24	Cytoskeleton component (Kabbani et al. 2007)
Protein kinase C	U33624	3.74	2.34	Serine/threonine phosphorylation (Kabbani et al. 2007)
creatine kinase	CL4483-1	1.35	0.49	interacting with mouse β2 (Kabbani et al. 2007)
Dynamin 1	CL181-1	−1.30	−0.72	nAChR trafficking (Kabbani et al. 2007)
Lysozyme C-1 precursor	CL305-1	−1.27	−0.17	interacting with mouse β2 (Kabbani et al. 2007)
	CL3823-2	−0.42	1.82	
	CL691-2	4.38	−0.58	
14–3–3 protein	CL1627-1	2.45	1.27	nAChR stabilization (Jeanclos et al. 2001)
	CL1627-3	−2.20	−0.56	
Synaptotagmin	CL2343-1	1.67	1.31	interacting with mouse β2 (Kabbani et al. 2007)
	CL298-2	−2.45	−0.93	

Table 5. The expression variations of 17 unigenes from transcriptome and qRT-PCR analysis

Unigene ID	LD (transcriptome)		CK		qRT-PCR(LD)		HD (transcriptome)		qRT-PCR(HD)	
	Log_2Ratio (CK-LD)	Fold change	Means	SEM	Means	SEM	Log_2Ratio (CK-HD)	Fold change	Means	SEM
CL2087-1	4.98	31.559	1.000	0.075	7.724	1.223**	2.25	4.757	6.236	0.870**
U5019	4.45	21.857	1.000	0.039	3.311	0.498**	3.90	14.929	5.557	1.144**
CL2702-3	4.06	16.679	1.000	0.112	10.460	2.250**	2.60	6.063	2.289	0.295**
U33624	3.74	13.361	1.000	0.085	5.802	1.416**	2.34	5.063	3.126	0.533**
CL2319-1	3.32	9.987	1.000	0.058	1.265	0.181	4.10	17.148	0.983	0.112
U13389	2.81	7.013	1.000	0.136	1.744	0.322*	2.43	5.389	1.138	0.116
CL2611-1	2.77	6.821	1.000	0.102	4.123	0.710**	2.34	5.063	2.790	0.428**
U21296	2.37	5.169	1.000	0.071	0.112	0.042**	2.24	4.724	0.308	0.060**
U29431	1.12	2.173	1.000	0.065	0.470	0.086**	0.59	1.505	1.117	0.085
CL4026-1	−7.30	0.006	1.000	0.032	0.029	0.007**	−1.92	0.264	0.359	0.048**
U6027	−3.69	0.077	1.000	0.114	0.016	0.004**	−1.42	0.374	0.812	0.071*
CL2853-2	−3.50	0.088	1.000	0.070	0.325	0.042**	−2.64	0.160	0.283	0.054**
U31578	−1.81	0.285	1.000	0.072	1.144	0.118	−1.21	0.432	0.952	0.117
U31293	−1.71	0.306	1.000	0.056	1.113	0.095	−2.52	0.174	0.864	0.093
CL181-1	−1.30	0.406	1.000	0.064	2.029	0.530**	−0.72	0.607	1.090	0.125
U38505	0.98	1.972	1.000	0.103	0.403	0.061**	−2.92	0.132	0.272	0.046**
CL3168-1	0.50	1.414	1.000	0.085	0.957	0.122	1.20	2.297	0.886	0.069

* Significantly different at 0.05 level; **significantly different at 0.01 level.

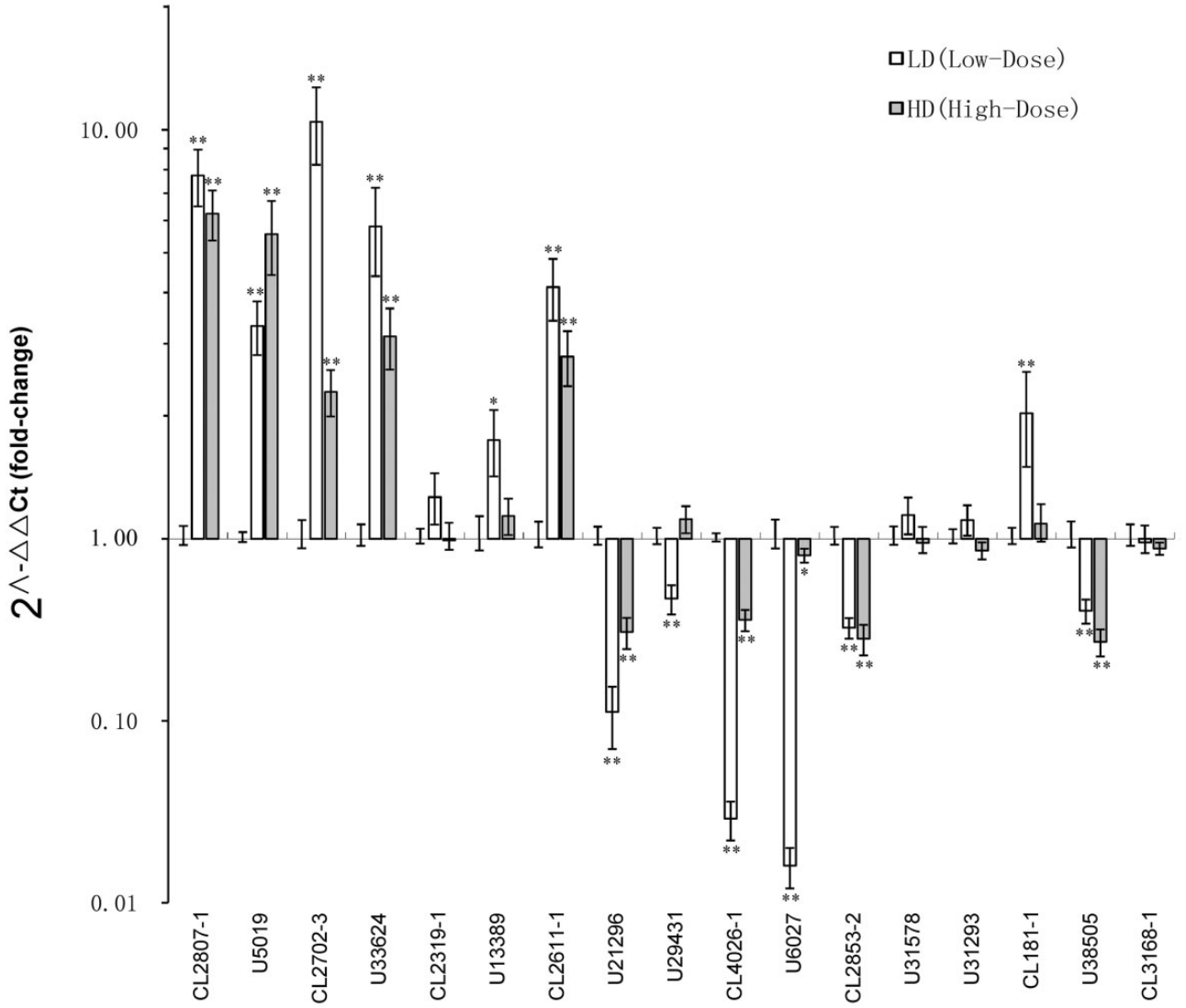

Fig. 1. The fold change in expression level of 17 unigenes between different dose treatments (LD and HD) and CK determined by qRT-PCR. The expression levels in LD and HD were normalized to that in CK. Values are plotted as means ± SE of at least three repeats. *Significantly different at 0.05 level; **Significantly different at 0.01 level.

In our study, 13 and 9 protein differentially expressed unigenes were identified in CYP6 subfamilies at LD and HD, respectively. The expression of most CYP6 unigenes was upregulated (Table 4) CYP6BQ9 in the CNS of *Tribolium castaneum* and CYP6D1 in the CNS of *Musca domestica* are involved in insecticide metabolism and so are associated with insecticide resistance (Korytko and Scott 1998, Zhu et al. 2010). Thus, the upregulation of these CYP6 genes in the locust CNS might be involved in variation in imidacloprid sensitivity.

A greater number of genes were regulated by LD than HD. The levels of regulation were also higher at LD than that at HD. This is consistent with a previous report that the expression of few metabolic enzyme genes was induced by high insecticide concentrations (Willoughby et al. 2006). Among 23 genes regulated at both LD and HD, the regulation levels of 19 P450 unigenes were higher at LD than at HD. Meanwhile, more CYP4 subfamily unigenes were upregulated than that downregulated at LD. In contrast, more unigenes were downregulated than that upregulated at HD. The response might be due to the induction of different regulatory mechanisms in response to sublethal and lethal insecticide doses. In response to sublethal dose, insects mainly try to generate the tolerance to insecticides via gene expression regulation. However, in response to a lethal dose, gene expression regulation is focused on survival.

Validation of Differential Gene Expression. To evaluate the validity of Illumina analysis, a total of 17 differentially expressed unigenes, including one nAChR subunit (Locα8), four nAChRs-interacting proteins unigenes, and 12 P450s unigenes were selected to test expression changes by qRT-PCR following imidacloprid treatments at LD and HD. The expression variation of these unigenes is shown in Table 5 and Figure 1. Among 17 differentially expressed unigenes from the transcriptome analysis, the expression changes in most (more than half) unigenes were confirmed by qRT-PCR in either LD or HD treatments, including upregulation and downregulation. These results indicated that the transcriptome analysis could generally, although not completely, reflect the changes of gene expression with some admissible error. The results of qRT-PCR also supported the conclusion that a greater number of unigenes were regulated by LD than that by HD. However, the changes of some unigenes were not validated by qRT-PCR, such as the downregulation of U21296 and U29431 in LD, which were upregulated in the transcriptome analysis. So, the results from transcriptome analysis always need validation from qRT-PCR or other methods.

In conclusion, the response to different imidacloprid doses differed significantly in locusts at the transcription level of related genes. The numbers and levels of nAChRs, interacting proteins, and P450 genes regulated by imidacloprid application were different between LD and HD treatments. Thus, different regulatory mechanisms would be motivated to mediate the response to stresses induced by different insecticide concentrations. Alternatively, it is possible that the expression variation of related genes could modulate neonicotinoid sensitivities by influencing insecticide detoxifications or by regulating target sensitivities, which might be potential factors for insecticide resistance.

SUPPLEMENTARY DATA

Supplementary data are available at *Journal of Insect Science* online.

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