SCIENCE CHINA
Life Sciences

THEMATIC ISSUE: Biotic information and pest control
• LETTER TO THE EDITOR •

August 2017 Vol.60 No.8: 931–934
doi: 10.1007/s11427-017-9104-5

Transcriptomic responses of three aphid species to chemical insecticide stress

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Received April 6, 2017; accepted May 5, 2017; published online July 21, 2017

Citation: Guo, K., Yang, P., Chen, J., Lu, H., and Cui, F. (2017). Transcriptomic responses of three aphid species to chemical insecticide stress. Sci China Life Sci 60, 931–934. doi: 10.1007/s11427-017-9104-5

Dear Editor,

Aphids (Hemiptera: Aphididae) are economically important pests that are distributed worldwide and cause serious economic losses through direct feeding on plant, plant virus transmission, and honeydew production (Zhang and Zhong, 1983; Blackman and Eastop, 2000). Control of these aphid species relies almost exclusively on the use of chemical insecticides, such as organophosphates, carbamates, pyrethroids, cyclodienes, and neonicotinods. A number of aphid species have become resistant to at least one type of insecticide (Simon et al., 2001; van Emden and Harrington, 2007; Silva et al., 2012 in Supporting Information). Most research efforts have focused on elucidating the molecular basis of inherited metabolic resistance in insecticide-resistant aphids (Hemingway, 2000; Choi et al., 2001; Sun et al., 2005; Cao et al., 2008a; Cao et al., 2008b; Puinean et al., 2010). Less attention has been given to the short-term transcriptome responses of insects to insecticides. Investigation of such short-term or stressed responses may lead to the discovery of a novel molecular mechanism involved in chemical stress that was undetected by standard toxicological studies.

In this study, we performed in-depth transcriptome analyses on three aphid species, namely, *Acyrthosiphon pisum*, *Aphis gossypii*, and *Aphis glycines*, exposed to organophosphorus monocrotophos, carbamate methomyl, or neonicotinoid imidacloprid. Twelve cDNA libraries were sequenced using the Illumina sequencing platform, and 22 million to 51 million clean reads were obtained (EMBL_EBI accession number: SRP062763). The Q30 score of each library exceeded 97 %, which indicates high sequencing quality (Table S1 in Supporting Information). A total of 53,876 unigenes with an average length of 888 bp for *Ap. gossypii* and 76,875 unigenes with an average length of 964 bp for *Ap. glycines* were assembled (Table S2 and Figure S1 in Supporting Information). The GC contents of these two aphid unigenes were similar, i.e., 35.6% and 33.8%, respectively. For *Ac. pisum*, around 70% of the reads from the four libraries mapped to the genome, and 19,830 genes were detected for expression (Table S2 in Supporting Information).

unigenes of *Ap. gossypii* (22,111, 41%) and *Ap. glycines* (25,160, 33%) were assigned with annotations in at least one database, i.e., NR, SwissProt, TrEMBL, InterPro, GO, or KEGG, using BLASTx algorithm with a $<10^{-5}$ cut-off *E*-value (Table S2 in Supporting Information). The *Ac. pisum* genes detected for expression were assigned

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with annotations of GO, KEGG, or InterPro. Among the NR-hit unigenes, 82% of *Ap. gossypii* unigenes and 89% of *Ap. glycines* unigenes matched to *Ac. pisum* sequences. Minority of unigenes showed the highest homology to genes of other insects (Figure S2 in Supporting Information). In addition, sequences of aphid obligate symbiont *Buchnera aphidicola* were also detected in the two aphid species. In terms of GO classification for the unigenes, the three aphid species showed similar pattern in most categories of biological process, cellular component, and molecular function (Figure S3 in Supporting Information). However, variation existed in several GO categories (Figure S4 in Supporting Information).

We exposed each aphid species to a diagnostic dose of each insecticide that results in 50% mortality. *Ap. gossypii* and *Ap. glycines* showed a similar pattern of gene expression change, which forms two groups. Three groups of gene expression change were observed in *Ac. pisum* (Figure 1A). The three aphids showed the most differently expressed unigenes (DEGs) (3,788) to methomyl and the least DEGs (2,987) to imidacloprid (Figure 1B).

In response to imidacloprid, genes with hydrolase activity and calcium ion binding were upregulated. Meanwhile, genes with small molecule binding, transferase activity, transaminase activity, and ion transmembrane transporter activity were downregulated in the three aphid species (Figure

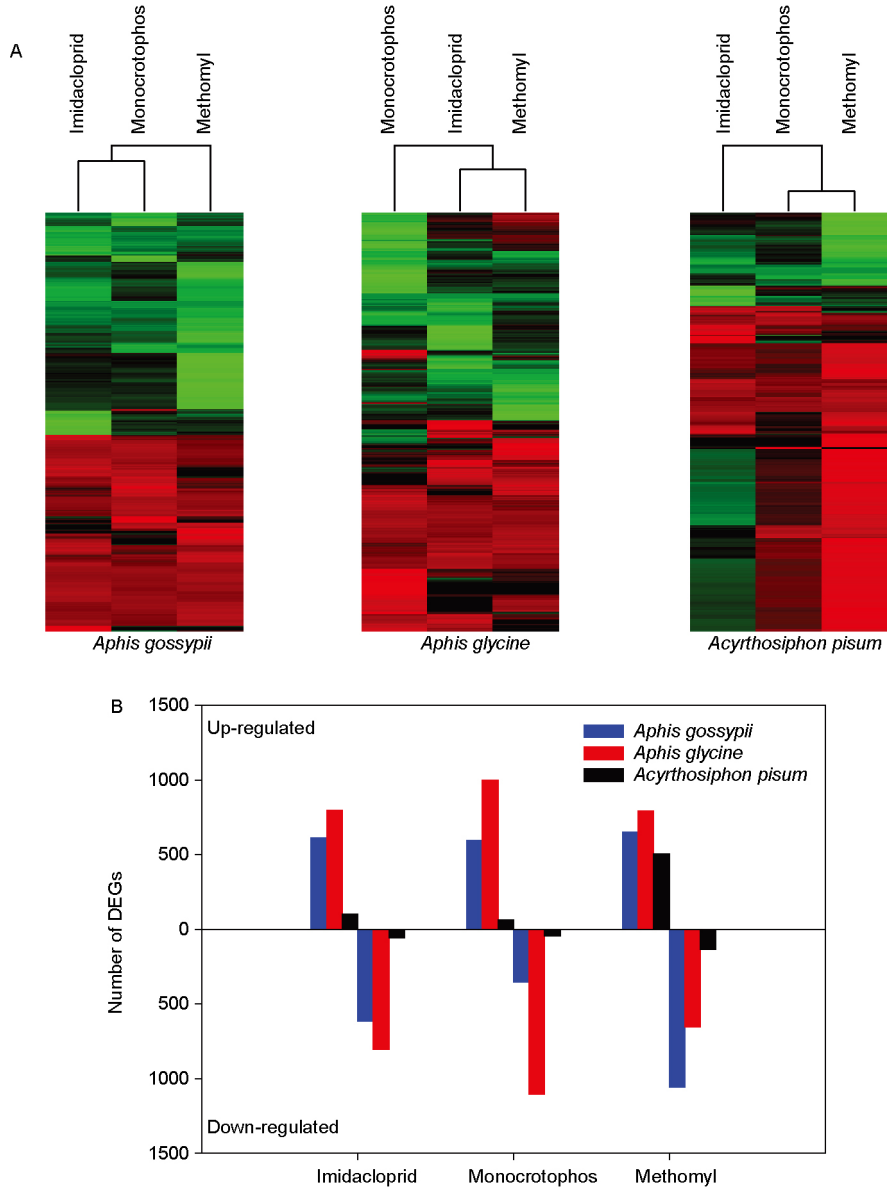

Figure 1 Transcriptional responses of three aphid species to three insecticides. A, Heat map and clustering anlaysis of expression profiles for all unigenes of aphids after imidacloprid, monocrotophos, and methomyl treatments. Each unigene is represented by a single row of colored boxes. Each insecticide is represented by a single column. The unigene values, represented by $\log_2$ (RPKM of insecticide/RPKM of control), are presented using various colors. Red represents higher expression values, and green represents lower expression values. B, Numbers of the differentially expressed unigenes (DEGs).

S5A in Supporting Information). The calcium ion-binding genes contained genes with WD repeat-containing protein. The transaminase activity genes contained aspartate aminotransferase genes. Five KEGG pathways, including ascorbate and aldarate metabolism (WD repeat-containing protein), caprolactam degradation (WD repeat-containing protein), glycerolipid metabolism (glycerate kinase or pancreatic lipase-related protein 2), MAPK signaling pathway (heat shock protein 70, poly U-binding-splicing factor half pint, or phosphatase 22-B), and pentose phosphate pathway (WD repeat-containing protein), were activated using imidacloprid in the three aphid species (Table S3 in Supporting Information).

After monocrotophos treatment, genes with hydrolase activity, transmembrane transporter activity (mainly ion transmembrane transporter activity), oxidoreductase activity that acts on paired donors with incorporation or reduction of molecular oxygen, protein dimerization activity, and zinc ion binding were upregulated in the three aphid species (Figure S5B in Supporting Information).

After methomyl treatment, genes with DNA binding, RNA binding, ATP binding, GTP binding, hydrolase activity that acts on ester bonds, monooxygenase activity, protein dimerization activity, transferase activity transferring acyl groups, and kinase activity were upregulated. Meanwhile, genes with ion transmembrane transporter activity were downregulated in the three aphid species (Figure S5C in Supporting Information). The upregulated monooxygenase genes were P450s. Three KEGG pathways were activated by methomyl in the three aphid species. These pathways include fat digestion and absorption (pancreatic lipase-related protein 2 or diacylglycerol o-acyltransferase), insulin signaling pathway (phosphoenolpyruvate carboxykinase, cytokine-inducible SH2-containing protein, phosphatase 1 regulatory subunit 3B, or MAP kinase-interacting serine/threonine-protein kinase 1), and MAPK signaling pathway (heat shock protein 70 or MAP kinase-interacting serine/threonine-protein kinase 1) (Table S3 in Supporting Information).

When viewed from the reaction of the three aphid species to the three insecticides, the aphids activated the expression of genes with hydrolase activity, transmembrane transporter activity, and zinc ion-binding functions under the stress of chemical insecticides. The genes with nucleic acid binding, protein binding, and oxidoreductase activity functions also responded to chemical insecticides.

Unigenes that encode putative detoxification enzymes of CCEs, P450s, and GSTs were identified in the three aphid species. *Ap. gossypii*, *Ap. glycines*, and *Ac. pisum* had 29, 56, and 29 CCE transcripts, 10, 12, and 20 GST transcripts, and 63, 117, and 83 P450 transcripts, respectively (Table S4 and Figures S6–S10 in Supporting Information). The CYP3 subfamily was only composed of CYP6 genes in the three aphids (Figure S9 in Supporting Information). In the 419 detoxification enzyme unigenes of the three aphid species, 38 (9%) changed the expression levels under the stress of chemical insecticides. Most of these unigenes were P450s and CCEs (Tables S5 and S6 in Supporting Information). The varied P450s were mainly CYP3 and CYP4 genes. The varied CCEs included A, E, and L classes. A and E classes of CCEs were downregulated, and L class of CCEs was upregulated.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

Acknowledgements *This work was supported by the National Natural Science Foundation of China (31272364, L1524009), Strategic Research of Chinese Academy of Science (2015-SM-C-02), and the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB11040200).*

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SUPPORTING INFORMATION

Table S1 Statistics of three aphid transcriptomes

Table S2 Assembly and annotation of the transcriptomes of the three aphid species

Table S3 Common KEGG pathways for the upregulated unigenes of the three aphid species after imidacloprid or methomyl treatment

Table S4 Number and classification of unigenes that putatively encodes for carboxylesterases (CCEs), glutathione S-transferases (GSTs), and P450 monooxygenases (P450s) in three aphid species

Table S5 Up-regulated unigenes that putatively encode for carboxylesterases (CCEs), glutathione S-transferases (GSTs), and P450 monooxygenases (P450s) in three aphid species after three insecticide treatments

Table S6 Down-regulated unigenes that putatively encode for carboxylesterases (CCEs), glutathione S-transferases (GSTs), and P450 monooxygenases (P450s) in three aphid species after three insecticide treatments

Figure S1 Length distribution of the *Aphis gossypii* (A) and *Aphis glycine* (B) unigenes.

Figure S2 Species distribution of the top BLAST hits of *Aphis gossypii* (A) and *Aphis glycines* (B) unigenes in the NR database.

Figure S3 Gene Ontology classifications (level 2) for the *Aphis gossypii* (A), *Aphis glycines* (B), and *Acyrthosiphon pisum* (C) transcriptomic unigenes.

Figure S4 Gene Ontology classifications (level 4) with a large difference in the number of transcriptomic unigenes among the three aphid species.

Figure S5 Gene Ontology classifications for the differentially expressed unigenes (DEGs) of the three aphid species after the imidacloprid (A), monocrotophos (B), or methomyl (C) treatment.

Figure S6 Phylogenetic tree of the carboxylesterases from three aphid species constructed with neighbor-joining method. The tree topology confidence was assessed through bootstrap analysis of 1000 replicates. Bootstrap values higher than 70% were present at the nodes. The number in bracket indicates the multiple unigenes with an extremely high similarity and in the same position of the tree. The unigenes colored with blue, red, and black are from *Aphis gossypii*, *Aphis glycines*, and *Acyrthosiphon pisum*, respectively. The differentially expressed genes under insecticide treatments are highlighted with stars.

Figure S7 Phylogenetic tree of glutathione S-transferases from three aphid species constructed with neighbor-joining method. Other information is similar to those presented in Figure S6. The differentially expressed genes under insecticide treatments are highlighted with stars.

Figure S8 Phylogenetic tree of the cytochrome P450 monooxygenases family 2 subfamily (CYP2) and the mitochondrial cytochrome P450 monooxygenases (CYPM) from three aphid species constructed with neighbor-joining method. Gene annotation was in parenthesis. Other information is similar to those presented in Figure S6. (A) CYP2. (B) CYPM.

Figure S9 Phylogenetic tree of the cytochrome P450 monooxygenases family 3 subfamily (CYP3) from three aphid species constructed with neighbor-joining method. Gene annotation was in parenthesis. Other information is similar to those presented in Figure S6. The differentially expressed genes under insecticide treatments are highlighted with stars.

Figure S10 Phylogenetic tree of the cytochrome P450 monooxygenases family 4 (CYP4) from three aphid species constructed with neighbor-joining method. Gene annotation was in parenthesis. Other information is similar to those presented in Figure S6. The differentially expressed genes under insecticide treatments are highlighted with stars.

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