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• LETTER TO THE EDITOR •

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Insecticide resistance status and detoxification enzymes of wheat aphids *Sitobion avenae* and *Rhopalosiphum padi*

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Dear Editor,

Wheat aphids are serious pests in wheat growing areas of China, and can lead to from 10% to over 30% reduction in wheat production (Wang et al., 2006). The main wheat aphids are *Sitobion avenae* (Fabricius), *Rhopalosiphum padi* (Linnaeus), *Metopolophium dirhodum* (Walker), and *Schizaphis graminum* (Rondani). In the Huang-Huai area of China, the dominant wheat aphid species are *S. avenae* and *R. padi*. For many years, farmers have applied chemical pesticides to control wheat aphids. The number of wheat aphids in north China has increased from year to year, probably due to the increased resistance to insecticides. In the insect-insecticide ecosystem, the most prevalent resistance mechanism is the metabolic resistance caused by elevated activities of detoxification enzymes carboxylesterases (CarEs), cytochrome P450 monooxygenases (P450s), and glutathione S-transferases (GSTs) (Rufingier et al., 1999; Puinean et al., 2010; Cui et al., 2015).

In order to evaluate resistance status, eight field populations of *S. avenae* and three populations of *R. padi* were collected from Heilongjiang, Hebei, Shandong, Henan, and Shaanxi provinces in 2012 (Table S1 in Supporting Information). Insecticide bioassays were performed to monitor resistance levels of these field populations of *S. avenae* and *R. padi* to organophosphate (phoxim and chlorpyrifos), carbamate (methomyl), neonicotine (imidacloprid), and pyrethroid (deltamethrin) insecticides (Tables S2 and S3 in Supporting Information). The dose-ortality curves of all field populations to the five insecticides were well represented by regression lines (P>0.05). For the eight field populations of *S. avenae*, although most populations had no significant resistance to all the five insecticides (P>0.05), a low but significant resistance was found in population TA (from Shandong province) to all the five insecticides (less than 3-fold resistance, P<0.05), in populations CZ and BD (from Hebei province) to imidacloprid and deltamethrin, and in population FQ (from Henan province) to methomyl and deltamethrin (less than 2-fold resistance, P<0.05). The highest resistance ratios were found in population TA to chlorpyrifos (2.22-fold) and to deltamethrin (2.14-fold). All three field populations of *R. padi* were sensitive to all five insecticises (P>0.05) except for a 1.36-fold resistance to deltamethrin for population HB (from Heilongjiang province). In a word, the populations of the two wheat aphid species showed only a low resistance or were susceptible to chemical insecticides.

The activities of the CarE, GST and P450 detoxification

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enzymes were examined in *S. avenae* and *R. padi* populations (Figure 1). For the eight field populations of *S. avenae*, only population TA showed an elevated CarE activity, i.e., 1.7-fold of the CarE activity in the susceptible strain. Significantly higher GST activities were observed in populations YL (from Shaanxi province) and HB (P<0.05). The P450 activities of all eight *S. avenae* field populations were higher to some extent compared to the susceptible strain, but only populations CZ and TA showed a significant increase (P<0.05), around 1.4-fold higher than the susceptible strain. For the three field populations of *R. padi*, there was no difference in CarE and P450 activities. A slight but significantly higher GST activity was observed in populations HB and FQ (P<0.05). For *S. avenae* populations, CarE enzyme activities were positively correlated with resistance levels to chlorpyrifos (r=0.841, P<0.01), deltamethrin (r=0.858, P<0.01), and methomyl (r=0.758, P<0.05). P450 enzyme activities were positively correlated with resistance levels to methomyl (r=0.746, P<0.05). No correlation was observed in *R. padi* populations (Figure S1 in Supporting Information).

The transcriptomes of *S. avenae* from population YL and *R. padi* from population HB were sequenced using Illumina Hiseq2000 (Table S4 in Supporting Information). After filtering low-quality sequences and adaptor contamination, approximately 54 M high-quality clean reads were obtained for each of the two libraries. The GC contents of *S. avenae*

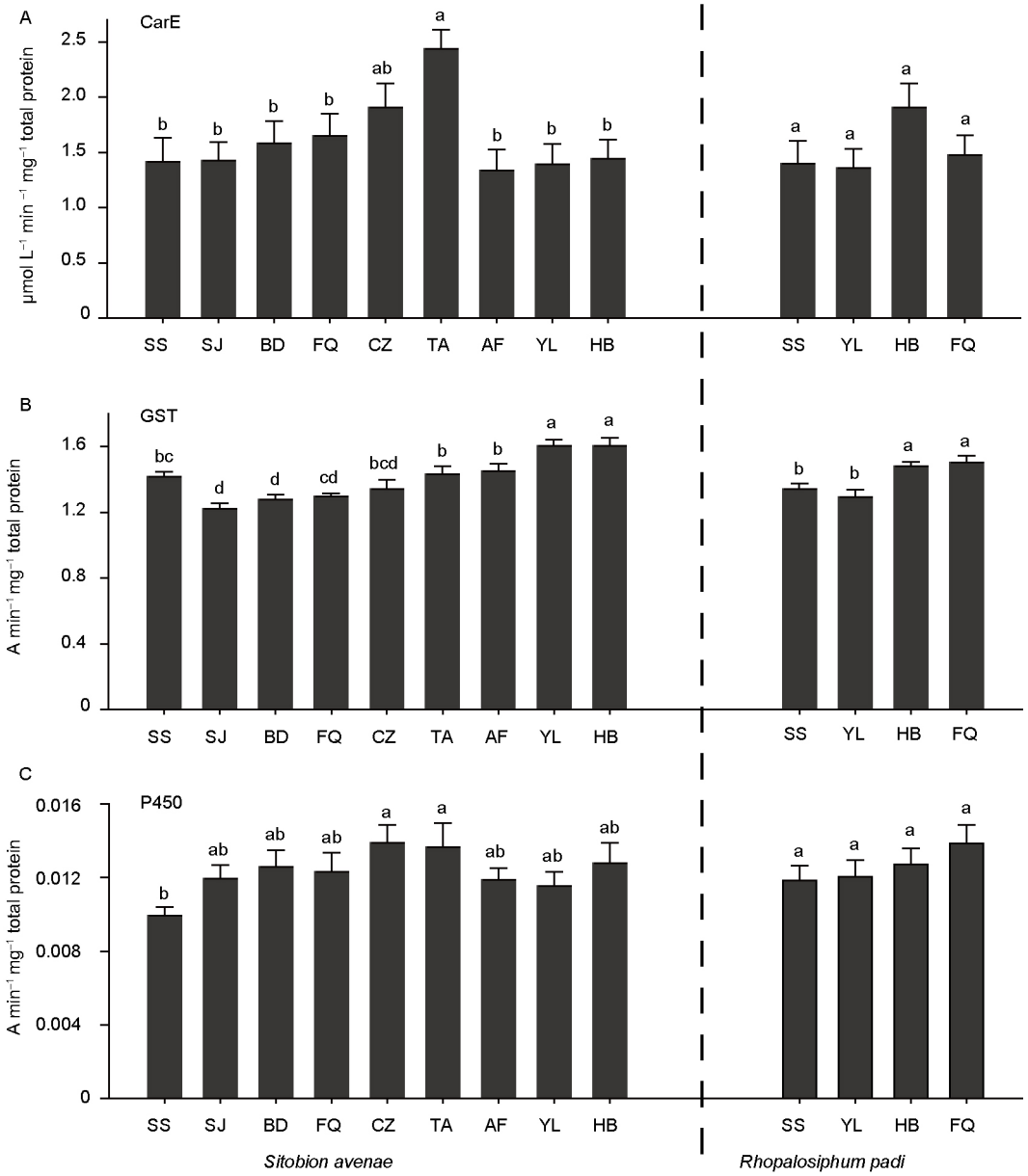

Figure 1 The activities of carboxylesterases (A), cytochrome P450 monooxygenases (B) and glutathione S-transferases (C) from field populations of *Sitobion avenae* and *Rhopalosiphum padi*. Different letters above columns indicate a significant difference among field populations evaluated by one-way ANOVA using SPSS17.0. SS, susceptible strain.

and *R. padi* transcripts were 44.9% and 40.9%, respectively, which were 6.1% and 2.1% higher than that of pea aphid transcripts at 38.8%. The sequencing data were deposited in the Sequence Read Archive (SRA, http://www.ncbi.nlm.nih.gov/Traces/sra) of National Center for Biotechnology Information (NCBI) with accession number SRP108464. A total of 42,200 unigenes with a mean length of 700 bp for *S. avenae* and 37,629 unigenes with a mean length of 522 bp for *R. padi* were *de novo* assembled. The unigene length distribution demonstrated that 9,612 unigenes (22.8%) of *S. avenae* were longer than 1,000 bp and 5,248 unigenes (14.0%) of *R. padi* were longer than 1,000 bp (Figure S2 in Supporting Information). Overall, 34,350 unigenes (81.4%) of *S. avenae* and 30,959 unigenes (82.3%) of *R. padi* were assigned with annotations in at least one database, i.e., NR, NT, SwissProt, COG, GO and KEGG (Table S4 and Figure S3 in Supporting Information).

A total of 146 detoxification enzymes, including 38 CarEs, 87 P450s, and 21 GSTs, were identified from *S. avenae*; and 110 detoxification enzymes, including 30 CarEs, 64 P450s, and 16 GSTs, were identified from *R. padi* (Table S5 in Supporting Information). Thus *S. avenae* possessed 33 % more detoxification enzymes than *R. padi*. According to Ramsey et al. (Ramsey et al., 2010), *A. pisum* had 132 homologous detoxification enzymes, which were used here as references for the phylogenetic classification of the detoxification enzymes from *S. avenae* and *R. padi*. For CarEs, six classes, representing dietary/detoxification (class A), hormone/semiochemical processing (class E), and neuro/developmental functions (classes I–L), were identified from the two wheat aphids (Table S5 and Figure S4 in Supporting Information). *S. avenae* had more CarEs involved in xenobiotic metabolism (class A) and neuro/developmental process than *R. padi*. The largest detoxification gene family in the three aphid species is the cytochrome P450 family, which was classified in four clades, i.e., CYP2, CYP3, CYP4, and the mitochondrial clade (Table S5 and Figures S5–S8 in Supporting Information). *S. avenae* had more CYP2, CYP3, and mitochondrial P450s than *R. padi*, especially for CYP3. The CYP3 clade in the three aphid species was only composed of CYP6 genes, and no CYP9 genes were retrieved. The GST family was more conserved between the two wheat aphids, even among the three aphid species, than the other two detoxification gene families (Table S5 and Figure S9 in Supporting Information).

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

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SUPPORTING INFORMATION

Table S1 Collection information of *Sitobion avenae* and *Rhopalosiphum padi* field populations in China

Table S2 Resistance observed in bioassays to five insecticides in eight populations of *Sitobion avenae* from China

Table S3 Resistance observed in bioassays to five insecticides in three populations of *Rhopalosiphum padi* from China

Table S4 Summary of the two wheat aphid transcriptomes

Table S5 Classification of detoxification gene families in *Sitobion avenae*, *Rhopalosiphum padi* and *Acyrthosiphon pisum*

Figure S1 Linear correlation between insecticide resistance levels and detoxification enzyme activities in *Sitobion avenae* populations.

Figure S2 Length distribution of unigenes from *Sitobion avenae* (A) and *Rhopalosiphum padi* (B).

Figure S3 *E*-value distribution (A), similarity distribution (B), and species distribution (C) of the BLAST hits of *Sitobion avenae* and *Rhopalosiphum padi* unigenes in the NR database.

Figure S4 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 presented at the nodes. The unigenes colored with red, blue, and black are from *Sitobion avenae*, *Rhopalosiphum padi* and *Acyrthosiphon pisum*, respectively.

Figure S5 Phylogenetic tree of the CYP2 clade of cytochrome P450 monooxygenases from three aphid species constructed with neighbor-joining method. Other information is same as Figure S4.

Figure S6 Phylogenetic tree of the CYP3 clade of cytochrome P450 monooxygenases from three aphid species constructed with neighbor-joining method. Other information is same as Figure S4.

Figure S7 Phylogenetic tree of the CYP4 clade of cytochrome P450 monooxygenases from three aphid species constructed with neighbor-joining method. Other information is same as Figure S4.

Figure S8 Phylogenetic tree of the mitochondrial cytochrome P450 monooxygenases from three aphid species constructed with neighbor-joining method. Other information is same as Figure S4.

Figure S9 Phylogenetic tree of glutathione S-transferases from three aphid species constructed with neighbor-joining method. Other information is same as Figure S4.

The supporting information is available online at http://life.scichina.com and https://link.springer.com. The supporting materials are published as submitted, without typesetting or editing. The responsibility for scientific accuracy and content remains entirely with the authors.