Differentially expressed microRNAs under imidacloprid exposure and identification in *Sitobion avenae*

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Abstract

Background: MicroRNAs (miRNAs), which are as short single-stranded non-coding RNAs, could regulate the expression of target genes, especially regulation or metabolism of endogenous or xenobiotic compounds. **Results:** The de novo assembly of the transcriptomes was obtained through Illumina short-read sequencing technology in *Sitobion avenae*. 57 miRNAs, of which 36 were known and 21 were novel were identified. Quantitative expression levels of miRNA showed that the expression of 5 miRNAs were significant up-regulation, and the expression of 11 miRNAs were significant down-regulation in the nymph of *S. avenae* treated by imidacloprid in comparison with the control, respectively. The candidate transcript target genes in *S. avenae* that could be regulated by these miRNAs were also carried out. The functions of the miRNAs, which could potentially regulate the target genes participated in the metabolism, regulatory or detoxification of *S. avenae* were clarified based on Gene Ontology and KEGG pathway. The effects of the miRNAs identified api-miR-1000, api-miR-316, and api-miR-iab-4 on susceptibility of *S. avenae* to imidacloprid was determined. The abundance of api-miR-1000, api-miR-316, and api-miR-iab-4 modulated by the addition of their own inhibitors to the artificial diet could change significantly the susceptibility of *S. avenae* to imidacloprid, which further proved that the regulatory affect of these miRNAs on regulation or metabolism of insecticides. **Conclusion:** It suggested that differentially expressed microRNAs under the stress of imidacloprid could play critical regulatory role in the resistance of *S. avenae* to imidacloprid.

Background

MicroRNAs (miRNAs) are between 18 and 23 nucleotides in length and have been shown to be responsible for the post-transcriptional regulation of mRNAs. miRNA could silence expression of target genes by complementarily binding to 5'/3' untranslated regions (UTRs) or coding sequences.¹⁻⁵ UTRs of target mRNAs were binded mainly through imperfect complementary base pairing.⁶ The exact match is considered to be related to target specificity.⁷

Studies have shown that miRNAs could regulate most of mammalian protein-coding gene,⁸ which could be critical mediators in a series of physiological and developmental pathways,⁹⁻¹² i.g apoptosis, cell proliferation, morphogenesis, and the metabolism of compounds.^{5, 13}

MiRNAs could mediate the detoxification/metabolism of xenobiotics through expression regulation of the detoxification genes, including nuclear receptors and xenobiotic-metabolizing enzymes,⁵ i.g human UDP-glucuronosyltransferase (UGT) 1A was proved to be mediated post-transcriptionally by mir-491-3p to metabolize raloxifene metabolites,¹⁴ human P450 CYP1A1 related to the metabolism of carcinogenic and metabolites can be mediated post-transcriptionally by miR-892a.¹⁵

Previous reports conducted in *Plutella xylostella* indicated that miRNAs could be involved in chlorantraniliprole resistance by regulating the expression quantity of UGT genes.¹⁶ *UGT2B7* and *UGT2B15* genes, which were proved to be mediated post-transcriptionally by miRNA;¹⁷ *Culex pipiens*

miRNAs are involved in the pyrethroid resistance by regulating the expression quantity of P450 genes.¹⁸
¹⁹ There are many miRNAs known involved in mediating the metabolic enzymes of xenobiotics in animals, while the affects of miRNAs on regulation of detoxification/metabolism of xenobiotics in pests is less understood.

The grain aphid, *Sitobion avenae*, is as the main pest of wheat distributed worldwide, which not only harms by sucking crop juice, but also spreads yellow dwarf virus disease, and leads to serious economic loss. The control of this aphid has depended largely on the use of chemically synthesized pesticides.²⁰ Imidacloprid is as one of neonicotinoids that targets sucking pests, such as aphids, green leaf bug and so on.²¹ It has been prevalently applied in wheat field to wheat aphid in China. So, *S. avenae* has developed serious resistance to imidacloprid.²²

Here, we performed high-throughput sequencing of short RNA libraries of control and imidacloprid treatment to detect differentially expressed microRNAs, and the functions of the miRNAs in regulating genes involved in the metabolism/ detoxification of *S. avenae* to xenobiotics. This will help understanding the roles of miRNAs in the regulation of the resistance to imidacloprid in insects.

Methods

2.1 Culture condition

The susceptible strain of *S. avenae* used was from the greenhouse for more 10 years, and has never been exposed to any insecticides. The culture condition was mentioned as previously.²³

2.2 Imidacloprid treatments

Leaf-dipping with aphids method was conducted with minor modifications as previously described Chen *et al.*²⁴ Leaf-dipping with 3rd instar nymphs aphids were immersed in the imidacloprid solution (0.5 mg L⁻¹) for 5-10 s. And their imidacloprid-treated counterparts treated with distilled water as the control were also collected. Surviving aphids (N = 6 for each sample), including imidacloprid challenge and counterparts were collected at the same time points (24 h).

2.3 RNA isolation, miRNA library construction and Illumina sequencing

Total RNA was extracted from controls and imidacloprid treatments of *S. avenae* using TRIzol reagent (Invitrogen, Shanghai, China) following the manufacturer's protocol and re-suspended in nuclease-free water. 1% agarose gels were monitored to determine if RNA extracted was degraded and contaminated, and then purity, concentration and integrity of RNA were measured by NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE), Qubit® RNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, CA, USA) and RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies,

CA, USA), respectively. Approximately 3 µg of total RNA as input material of each sample was used for constructing the small RNA library and conducted in Novogene, Beijing, China as previous description²⁵.

2.4 Bioinformatics analysis, miRNA prediction, and miRNA target prediction.

S. avenae miRNAs, were confirmed by miRDeep2 software.²⁶ Raw reads from the 4 libraries, including imidacloprid treatments and controls (2 biological replicates of each sample), were sent as insert into miRDeep2, and then the data (each library) was further separately analyzed. The default options and settings were used to performed the miRDeep2 analysis. And the sequences with polyA tails of the raw reads, the length of miRNAs ranging from 18 to 30 nt was selected for further being analyzed after the adaptor sequences was trimmed, and removed snRNA, rRNA, snoRNA and tRNA. The sequences, mapping to the mature miRNAs in the premiRNAs and miRBase, and mapping to the *Acyrtosiphon pisum* genome would be identified to be as known mature miRNAs. The clean reads identified of *S. avenae* transcriptome has been sent to NCBI/SRA database, the accession number was SRP182781. Miranda performed the target genes prediction of miRNA.²⁷ The putative mRNAs that targeted by miRNAs were predicted against *S. avenae* transcriptome nucleotide sequences. 2 software programs were conducted for miRNA target prediction, miRanda²⁸ and RNAhybrid.²⁹ The predicted miRNA target genes would be selected for further analysis. And then the target genes predicted would be aligned by the BLASTX program of NCBI, as well as mapping and annotation of the gene sequences were conducted using BLAST2GO.³⁰ The Gene Ontology (GO) database, Genomes (KEGG) pathway and Kyoto Encyclopedia of Genes were used to further analyse putative identified target genes of miRNAs.

2.5 Differential expressed miRNAs between controls and imidacloprid treatments of *S. avenae*

In order to obtain the miRNAs of aphids affected by imidacloprid, the expression level of miRNAs was performed between controls and treatments of *S. avenae*. The edgeR software (3.10.2) obtained from Bioconductor version 3.1 was used to analyze the read counts of identified miRNA.³¹

The P-values was calculated by the Benjamini-Hochberg method.³² P-value corrected was set at 0.05 as the threshold for expressed difference by default significantly. Normalization of miRNA counts between libraries of controls and treatments was executed according to the total number of reads across libraries in silico. Normalized expression = Actual miRNA count/Total count of clean reads×10⁶. A false discovery rate (FDR) of the miRNAs <0.05, and a fold change ≥2 was deemed to be significant.

2.6 Quantitative real-time PCR (qPCR) validation

To validate the miRNA data obtained by the deep sequencing, 3 miRNAs were selected to confirm their expression by qPCR. Total RNA, first strand cDNA synthesized, SYBR Green Master Mix (miScript SYBR Green PCR Kit, Qiagen) and qPCR procedure determined was described as previously.³³ Relative expression quantification was calculated by the 2^{-ΔΔCt} method.³⁴ U6 snRNA was used to normalize expression quantiy of miRNAs as an endogenous control. The primers designed were showed in Table 1.

2.7. MiRNA inhibitor feeding *in vitro*, and subsequent impacts on imidacloprid susceptibility

The rearing method and the artificial diet used were as previously described.³⁵ with minor modifications. Both ends of transparent glass tube were open, sterilized and used as the feeding device (4 cm in length, 2.5 cm diameter). 25% sucrose as artificial diet was fit into 2 layers of parafilm sealed in the one end of the feeding device, and then healthy apterous adults were transferred to the device for rearing covered by the mesh to prevent them from escaping. The aphids were reared under the condition described previously. Each sample contained three replications.

To evaluate the inhibitory effect of miRNAs after the feeding of miRNA inhibitors, the artificial diet with miRNA inhibitors at a 2.5 mM/L final concentration, 50 healthy apterous aphids were placed to the artificial diet mixed with miRNA inhibitor, and the NC-inhibitor (a negative control) were used as the control; Following the feeding of 24 h, the aphids survival were collected for RNA extraction, 3 replicates were carried out.

To evaluate the affects of miRNAs modulated on sensitivity of *S. avenae* to imidacloprid, 50 healthy apterous adults were placed to the artificial diet that contained imidacloprid (0.1 mg/L) with a 2.5 mM/L final concentration miRNA inhibitor, the NC-inhibitor (a negative control) were used for the control. 3 replicates were carried out, and mortality was caculated at 48 h. The miRNA inhibitors used were provided by Sangon Biotech Co., Ltd (Shanghai, China).

Results

3.1 Illumina sequencing data analysis

There were 14, 841,584, and 13, 964, 533 raw reads, identified from RNA libraries from the imidacloprid treatment and control (Table 2). The length of *S. avenae* sRNAs was between 18 nt and 35 nt, comprising 5.28% and 5.08% of the all reads with 22 nt size from the imidacloprid treatment and control (Fig. 1A). After discarding low quality sequences and adapter, The unique reads, 6,992,451 (47.11% of total) and 6,349,363 (45.47%) remained from the control and imidacloprid treatments libraries, respectively (Table 2). The distribution in length of unique reads filtered indicated a distribution in the 2 libraries (Fig. 1B), where constituted 3.75% and 3.72% of the total reads at 22 nt, respectively, where at 23 nt constituted 5.18% and 6.23% of the total reads, respectively. Where in the maximum at 32 nt constituted 8.59% of the all reads of imidacloprid libraries, at 34 nt constituted 9.76% of the all reads of controls, which probably showed piRNA-like sRNAs in *S. avenae*. A little bit sRNAs had over a thousand reads, however most had less than 10 copies (Fig. 1C). sRNAs in *S. avenae* showed a significant bias at 5' (96.99%, and 97.51%, respectively) and 3' ends (53.38%, and 55.32%, respectively) in both libraries (Fig. 2) for the nucleotide U. .

3.2 Sequence and predication of miRNAs

According to the distribution in length of the reads, of sRNA libraries miRNAs at 22 nt accounted for 43.86% of all species of miRNAs (Fig. 1D). The query of the reads of putative miRNAs in *S. avenae* to miRNAs from *A. pisum* genomes in miRBase, the results showed 36 sequences conserved were identified

(Table S1). In addition, the query of the reads filtered in *S. avenae* against mature miRNAs, showing that 26 miRNA sequence families were identified from the miRBase database (Fig. 3A). It is predicted that the miR-10 family has the largest members ($n = 415$), next let-7 ($n = 401$), and miR-2 ($n = 180$). Insects were shared with these miRNAs, with the majority of miRNAs shared with the known *A. pisum* miRNAs, while a few extent miRNAs trended for other insect orthologs (Fig. 3B).

There were a total of 21 novel miRNAs potentially obtained, and named the prefix 'PC' (predicted candidate) based on the adopted nomenclature (Table S2). These specific miRNAs could be mapped to *S. avenae* transcriptome sequences. It was predicted from Mfold that stem-loop hairpin secondary structures (<18 kcal/mole³⁶) were consist of miRNAs precursor sequences, and some predicted structures in *S. avenae* were shown in Fig. S1.

Differentially expressed miRNAs between imidacloprid treatments and controls were also determined according to normalized differences of Illumina read counts, 57 *S. avenae* miRNAs, 16 of which (28.1%) were differential expression, including 11 down-regulation and 5 up-regulation (Table 3).

The PCR amplified products of api-miR-1000, api-miR-316, and api-miR-iab-4 indicated the expected size between 60 and 100 bp (a single band). Subsequent analysis of qPCR results confirmed that the expressed level of Api-miR-1000 was 5.5- fold higher in imidacloprid treatments compared to controls. While the expression of Api-miR-316, and Api-miR-iab-4 were 0.09-, and 0.12- fold lower in imidacloprid treatments compared to controls, respectively. (Fig. S2). Overall, qPCR results of miRNAs were indicated similar ranging trends as that of the RNA-seq analyses.

3.3 Target prediction of miRNAs.

The 16 differentially expressed miRNAs in *S. avenae*, the hypothetical targets within the UTRs of transcripts from *S. avenae* for the miRNAs were predicted using Target Scan software (Table S3). Possible functions predicted of these hypothetical target genes were annotated by GO enrichment, which could be involved in molecular functions, biological processes and cellular components, like development processes (Table S4). There were 58 GO terms, which were identified for predicted miRNA target genes according to GO level 2 (Fig. 4A), like the genes participated in metabolic processes.

These genes including 71 metabolic pathways predicted by KEGG enrichment analysis might be targeted by miRNA regulation (Fig. 4B). The transcript targets were predicted to orthologs of related insects, which showed that differentially expressed miRNAs between the control and the imidacloprid treatment in *S. avenae* could bind and promote the post-transcriptional regulation, including different biological pathways like xenobiotic metabolism (Table S5).

To confirm the potential function of miRNAs in the resistance of *S. avenae* to imidacloprid, the predicted target genes for the miRNAs that could participate in xenobiotic metabolism were further focused. Interestingly, target genes for some miRNAs were found to play critical roles in insects response to xenobiotics, like cytochrome P450s, UDP-glucuronosyltransferase, glutathione S-transferase, etc. (Table

4). Some miRNAs predicted have a lot of putative target genes, and many putative target genes were regulated by multiple miRNAs. (Table S3).

3.4 Modulation of miRNAs consequently impacts the susceptibility of *S. avenae* to imidacloprid

The expression levels of miRNAs, api-miR-1000, api-miR-316, and api-miR-iab-4, was significantly depressed in *S. avenae* aphids fed miRNA inhibitors compared to the quantity in *S. avenae* fed on NC-inhibitor and DEPC-water as controls (Fig. 5A). Mortality decreased significantly 21.73% in *S. avenae* aphids fed api-miR-1000 inhibitor under imidacloprid exposure compared to the controls. While the mortality increased significantly by 26.75% and 21.86% in *S. avenae* aphids fed api-miR-316, and api-miR-iab-4 inhibitors under imidacloprid exposure compared to the controls, respectively (Fig. 5B).

Discussion

It is predicted miRNAs can regulate a lot of protein-coding genes.³⁷⁻³⁹ And it is demonstrated that miRNAs are involved in regulation of various biological processes. In this study, miRNAs in *S. avenae* that might play regulatory roles in target genes responses to imidacloprid were identified and assessed. The identification and functional analysis of miRNAs differentially expressed in imidacloprid treated *S. avenae* were conducted, which may provide potentially understanding the regulation/detoxification of imidacloprid in insects. So far, the sequencing of small RNA has been as a widely trial way to obtaining miRNAs from a series of organisms based on sequencing technology of high throughput. Li et al.⁴⁰ found out 345 miRNAs from the winged and wingless *S. avenae*. While we only identified 57 miRNAs in the control and imidacloprid treatments of *S. avenae*. However, our sequencing data could also greatly enlarge the range of the available information and provide critical basis for further studying miRNAs related to insecticide resistance in *S. avenae*.

Our analysis on expression of read counts indicated that 16 miRNAs differentially expressed were identified between the imidacloprid treatment and control of *S. avenae*, significantly indicating that imidacloprid could modulate expression of miRNAs, suggesting a probable regulatory role of these miRNAs in the detoxication/metabolism of insecticides in *S. avenae*. Several up/down-regulated miRNAs in the imidacloprid treatment of *S. avenae* could be involved in the regulation of the metabolism of imidacloprid. i.g api-miR-1000, api-miR-316, and api-miR-iab-4 were up/down-regulated in the imidacloprid treatment of *S. avenae*, which suggests that they could be involved in the regulation of the metabolism of imidacloprid. *Drosophila melanogaster* miR-1000 of activity-dependent expression could indicate a mechanism of allowing neuronal activity to fine-tune the strength in the excitatory synaptic transmission, and regulate the expressed level of vesicular glutamate transporter, and load the glutamate into synaptic vesicles,⁴¹ *D. melanogaster* dme-miR-1000 was located in the intron of musashi,⁴² encoding an RNA-binding protein to regulate gene translation by prior expression in the nervous system;⁴³ Honeybee miR-1000 could be involved in musashi function of the nervous system,⁴⁴ ame-miR-1000 was identified first, which was expressed highly selective manner of a insect brain. 1 cytochrome P450 and 2 glutathione S-transferase genes could be recognized by mse-miR-316;⁴⁵ The accumulation endogenous

Ubx protein could be attenuated as miR-iab-4-5p ectopic expression, and a classical homeotic mutant phenotype could also be induced (the transformation of halteres into wings),⁴⁶ *Drosophila* dme-miR-iab-4 and dme-miR-iab-8 as miRNAs of bithorax-complex (BX-C) HOX bunches could participate in regulation of reproduction and neural patterning through suppressing HOX gene targets.⁴⁷

In order to further exploring the role of miRNAs in the regulating metabolism of imidacloprid, the hypothetical target genes for miRNAs identified were predicted, and many of them annotated could participate in a lot of biological processes. At the same time, several miRNAs from different families target genes were proved to be involved in the xenobiotic detoxication/metabolism.⁴⁸⁻⁵¹ We also found that several of the miRNAs targeting the genes involved in xenobiotic detoxication/metabolism were expressed differentially between the imidacloprid treatment and control of *S. avenae* by combining our analysis of differential expressed miRNAs with the prediction of the target genes.

The mortality of *S. avenae* exposed to imidacloprid significantly decreased after their feeding api-miR-1000 inhibitor. While the mortality of *S. avenae* exposed to imidacloprid significantly increased after their feeding api-miR-316, and api-miR-iab-4 inhibitors, respectively. This showed that miRNAs differential expressed may participate in metabolism of imidacloprid by modulating gene expression related to xenobiotic metabolism.

In conclusion, of 57 miRNAs obtained from *S. avenae*, the transcript levels of 16 miRNAs were ranged in *S. avenae* treated by imidacloprid. The prediction of targeting the genes related to xenobiotic metabolism showed that differentially expressed miRNAs could participate in the detoxication/metabolism of imidacloprid, and this represents a crucial resource of new small RNA genomics for further study in *S. avenae*.

Abbreviations

miRNAs: MicroRNAs; UGT: UDP-glucuronosyltransferase; qPCR: Quantitative real-time PCR; BLASTX: Similarity search of the NCBI protein database using a translated nucleotide query; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; nr: NCBI non-redundant protein sequences database; nt: NCBI nucleotide collection; UTRs: untranslated regions; SRA: Sequence Read Archive

Declarations

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Availability of data and materials

The transcriptome clean reads and computationally assembled sequences from this study were submitted to the NCBI/SRA database, under accession number: SRX374716.

Author contributions statement

S.Z and X.C conceived and designed the experiments, B.Z., and J.L. performed the experiments, B.Z., and S.Z. analyzed the data and wrote the manuscript text. J.L., and L.L. participated in the data analysis. All authors reviewed the manuscript.

Competing interest

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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Additional information

Table S1. Summary of conserved miRNA.

Table S2. Summary of predicted miRNA.

Table S3. The predicted targets for miRNAs in *Sitobion avenae*.

Table S4. GO annotation for identified *Sitobion avenae* miRNAs.

Table S5. KEGG analysis for targets of differentially expressed *Sitobion avenae* miRNAs.

Fig. S1. Predicted stem-loop structures for three novel miRNA precursors.

Fig. S2. qPCR validation of miRNAs potentially involved in resistance of *Sitobion avenae* to imidacloprid.

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Tables

Table 1. Primers were used in the study

Primer name	Sequence (5'-3')	Size/bp	Application
api-mir-1000	F:AGAGCACGATATTGTCCTGTCACA	73	qPCR
	R:TATGGTTGTTTCACGACTGGTTCAC		qPCR
api-mir-316	F:TTTGGTGTCTTTTCCGCTTTG	69	qPCR
	R:GTGCAGGGTCCGAGGT		qPCR
api-mir-iab-4	F:AGGCGAAGCGACGTATACTAAATG	68	qPCR
	R:GTGCAGGGTCCGAGGT		qPCR
U6	F:CAGCACATATACTAAAATTGGAACG	76	qPCR
	R:ACGAATTTGCGTGTCTATCC		qPCR
MircoRNA inhibitor N.C	CAGUACUUUUGUGUAGUACAA	21	miRNA function
api-mir-1000 inhibitor	UACUGCUGUGACAGGACAAUUAU	22	miRNA function
api-mir-316 inhibitor	CGGCAGCAAAGCGGAAAAAGACA	23	miRNA function
api-mir-iab-4 inhibitor	UCAGGAUACAUUUAGUAUACGU	22	miRNA function

Table 2. Distribution of miRNA reads from control and imidacloprid treatment of *Sitobion avenae*

Parameter	Controls	Imidacloprid treatments
Raw reads	14,841,584	13,964,533
Clean reads	14,224,837	13,492,898
3ADT and length filter	10,959,929	9,305,973
rRNA	253,777	247,747
tRNA	16	23
snoRNA	9,443	7,823
snRNA	25,993	24,246
Mappable reads	6,992,451	6,349,363

Note: 3ADT: reads that were removed as 3ADT was not found and length was less than 15nt and was more than 35nt. Mappable reads: reads from the raw reads that were passed by many the digital filters .

Table 3. The differentially expressed miRNAs in small RNA libraries of *Sitobion avenae*

miR_Name	Imidacloprid treatment_FPKM	Control_FPKM	P-value	Log ₂ ^(Fold Change)	Difference
api-miR-998	4021.337154	14561.84926	0	-1.8564	Down
Novel-28	435.4314115	2094.17899	1.93E- 286	-2.2659	Down
api-miR-306	691.567536	2386.390012	1.54E- 246	-1.7869	Down
Novel-22	742.7947608	1801.967968	1.83E- 120	-1.2785	Down
Novel-9	717.1811484	1704.564294	1.79E- 110	-1.249	Down
api-miR- 3047	332.9769618	779.2293917	3.01E- 50	-1.2266	Down
Novel-25	256.1361244	657.4747992	6.09E- 49	-1.36	Down
api-miR- 1000	128.0680622	0	8.16E- 28	8.0008	Up
api-miR-316	0	48.70183698	2.37E- 13	-6.6059	Down
Novel-39	51.22722489	0	4.65E- 13	6.6788	Up
Nove-35	153.6816747	48.70183698	2.31E- 12	1.6579	Up
api-miR- 263b	0	24.35091849	1.57E- 07	-5.6059	Down
api-miR- 2796	0	24.35091849	1.57E- 07	-5.6059	Down
api-miR-iab- 4	0	24.35091849	1.57E- 07	-5.6059	Down
api-miR- 3015a	25.61361244	0	2.52E- 07	5.6788	Up
api-miR- 3042	76.84083733	24.35091849	7.05E- 07	1.6579	Up

Table 4 MiRNAs target for putative genes related to xenobiotic metabolism in *Sitobion avenae*

miR_Name	Target genes related to xenobiotic metabolism
api-miR-998	S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase
novel-28	CYP3A19, glutathione S-transferase omega-1, glutathione S-transferase, C-terminal domain
api-miR-306	Microsomal epoxide hydrolase, glutathione S-transferase, C-terminal domain, P450 (GC vitamin D-binding protein), CYP4C1, CYP4V, CYP9Z4
novel-22	alcohol dehydrogenase 4, UDP-glucuronosyltransferase 2B10, UDP-glucuronosyltransferase 2B7, Cytochrome P450 (piezo-type mechanosensitive ion channel component 2), CYP4C1, Glutathione S-transferase (EEF1G elongation factor 1-gamma), CYP6A14
novel-9	UDP-glucuronosyltransferase 2C1, UDP-glucuronosyltransferase 1-7C UDP-glucuronosyltransferase 2B17, Glutathione S-transferase Mu 1, Glutathione S-transferase (phosphatidylinositol-binding clathrin assembly protein LAP), CYP4g15, CYP4C1
api-miR-3047	UDP-glucuronosyltransferase 2C1, carbonyl reductase 1, Microsomal glutathione S-transferase 1, UDP-glucuronosyltransferase 1-7
novel-25	UDP-glucuronosyltransferase 2C1, UDP-glucuronosyltransferase 1-7, CYP315A1, CYP2U1, P450 (piezo-type mechanosensitive ion channel component 2)
api-miR-1000	alcohol dehydrogenase class-3, S-(hydroxymethyl)glutathione dehydrogenase / alcohol dehydrogenase, glutathione S-transferase, C-terminal domain, CYP3A19,
api-miR-316	UDP-glucuronosyltransferase 2B7, UDP-glucuronosyltransferase 2B10, UDP-glucuronosyltransferase 351A3, Glutathione S-transferase (phosphatidylinositol-binding clathrin assembly protein LAP), Glutathione S-transferase (failed axon connections), Cytochrome P450 (piezo-type mechanosensitive ion channel component 2), CYP4C1, CYP6A2, CYP6A14
novel-39	UDP-glucuronosyltransferase 2B1, Cytochrome P450 (piezo-type mechanosensitive ion channel component 2), CYP4C1, CYP305a1, Cytochrome bd terminal oxidase
novel-35	Glutathione S-transferase GSTZ1
api-miR-263b	Glutathione S-transferase (phosphatidylinositol-binding clathrin assembly protein LAP), Cytochrome P450 (piezo-type mechanosensitive ion channel component 2), CYP49A1, CYP6A14, CYP315A1, CYP4C1, CYP6A13, CYP6J1
api-miR-2796	UDP-glucuronosyltransferase 2C1, UDP-glucuronosyltransferase 2A1, glutathione S-transferase C-terminal domain, CYP6A13, CYP4C1, CYP4V2, CYP49A1,
api-miR-iab-4	UDP-glucuronosyltransferase 2B18
api-miR-3015a	CYP314A1, CYP4C1, CYP6A13, CYP3A19
api-miR-3042	alcohol dehydrogenase class-3, UDP-glucuronosyltransferase 2A1dehydrogenase, glutathione S-transferase C-terminal domain, Glutathione S-transferase (phosphatidylinositol-binding clathrin assembly protein LAP), Cytochrome P450 (piezo-type mechanosensitive ion channel component 2), CYP3A19,
	Glutathione S-transferase (EEF1G elongation factor 1-gamma)

Figures

Figure 1

Characterization of miRNA sequences from *Sitobion avenae*.

Figure 2

Nucleotide bias of predicated miRNAs from *Sitobion avenae*.

Figure 3

MiRNAs characterization of *Sitobion avenae*.

Figure 5

Effects of miRNA modulation on susceptibility of *Sitobion avenae* to imidacloprid.

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