Transcriptome and expression analysis of Sogatella furcifera (Horváth) (Hemiptera: Delphacidae) in response to cycloxaprid

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Abstract

Background

The white backed planthopper, Sogatella furcifera (Horváth), causes substantial damage to many crops by direct feeding or transmitting plant viruses, especially southern rice black-streaked dwarf virus (SRBSDV) that has become a great threat to rice production in East Asia. Cycloxaprid, a novel cis-nitromethylene neonicotinoid insecticide, has good industrialization prospects because of its high efficiency against rice planthoppers, including imidacloprid-resistant populations. This chemical would be used extensively in future. However, very little is known about the influence on S. furcifera after cycloxaprid application at the molecular level. We sequenced S. furcifera transcriptome of female adult of S. furcifera using the Illumina sequencing.

Results

By de novo transcriptome assembling and massive parallel pyrosequencing, we constructed two transcriptomes of S. furcifera and profiled the alternation of gene expression in response to cycloxaprid treatment in transcriptional level. Over 157,906,456 nucleotides and 131,601 different unigenes were generated using Illumina technology from both cycloxaprid-treatment and no-treatment S. furcifera. And a total of 38,534 unigenes matched known proteins in at least one database, accounting for 29.28% in total unigenes. The number of Coding DNA Sequence (CDS) were 28,546 and that of the amino acid sequence of coding region were 22,299. A total 15,868 simple sequence repeats (SSRs) were identified. The trinucleotide repeats accounted for 45.1% (7,157) in total SSRs and the (AAG/CTT)n was the most frequent motif. There were 359 differentially expressed genes (DEGs) that might be induced by cycloxaprid. There were 131 genes up-regulated and 228 down-regulated. 22 unigenes may take participate in the resistance to cycloxaprid, such as cytochrome P450, Glutathione-s transferase (GST), Acid phosphatase (ACP), cadherin, etc.

Conclusions

Our study will provide a splendid database for future investigations of the resistance mechanism induced by cycloxaprid and will provide new strategies for pest management and crop protection.

Background

Cycloxaprid, which firstly reported in 2008, patented and developed in China and first named in 2011[1-3]. Its action mode was known as targeting insect nicotinic acetylcholine receptors (nAChRs) and was similar to that of imidacloprid and nitenpyram but cycloxaprid has already been proven more effective than three other neonicotinoids: imidacloprid, thiamethoxam and nitenpyram for the control of piercing and sucking insect pests (aphids, leafhoppers, whiteflies, planthoppers etc), especially *Sogatella furcifera* in future[4][5]. As an oxabridged cis-configuration neonicotinoid insecticide, cycloxaprid has been considered a substitute for imidacloprid, especially the management of imidacloprid-resistant insects because it has performed well in controlling a broad spectrum of insect pests and has low toxicity for mammals[6][7].

S. furcifera (Horváth), the whitebacked planthopper, is one of most destructive migratory pests around Asia [8-10]. Its distribution areas include south and south-east Asia countries, China, korea, Japan, northern Australia and south Pacific Islands[11][12]and causes damage by directly feeding and ovipositing on rice stems. Moreover, it is an important vector transmitting several rice viruses, especially the southern rice black streaked dwarf virus (SRBSDV) causing enormous loss of rice yield [13-17].

The ecological , toxicological and physiological perspectives of *S. furcifera* and other hemipteran insect pests, such as *Nilaparvata lugens*, *Laodelphax striatellus* and *Bemisia tabaci* have been extensively studied[18-24]. Transcriptomes of *Nilaparvata lugens* and *Laodelphax striatellus* were reported using next-generation DNA sequencing techniques[25][26], and that responsed to SRBSDV[16]and to high and low doses of cycloxaprid[4] had also been studied. Furthermore, the olfactory receptor gene family[27] and the salivary glands[28] of *S. furcifera* had been sequenced, ect. The possible factors related to cycloxaprid-resistance and related genes by insecticide applications had been conducted. By treated with a low-dose and a high-dose treatment, these findings, based on transcriptome, showed that CYP4, CYP6, and GSTd contributed significantly to insecticide detoxification resistance[4]. However, it is little known to the whole transcripts and unigenes change after treated with cycloxaprid, In this study, hence, we constructed two transcriptomes of *S. furcifera* and profiled the alternation of gene expression in response to

cycloxaprid induction at the transcriptional level. As a whole, 131,601 unigenes have been identified and 359 DEGs have been discovered.

Results

Bioassay

By bioassay, the LC10 and LC50 concentration of cycloxaprid-treatment was established. Regression equation was y= 0.97x + 3.9462 ($\chi 2$=0.814, R=0.9835, P=0.6656>0.05) , LC10= 0.5823 mg(L-1 [95% confidence interval (CI), 0.1289-1.2322] and LC50= 12.2011 mg(L-1 [95% CI, 6.4689-37.1984].

RNA isolation

The values of Optical Density (OD260/280) ranges from 1.962 to 2.005, the RNA integrity number (RIN) value of six samples were larger than 7.0, indicating that they were pure and not degraded. So, the Ribonucleic Acid (RNA) quality of all samples meet the requirements of sequencing.(Table 1)

Transcriptome Assembly of *S. furcifera*

The *S. furcifera* transcriptomes were generated from 5 female adults emerged in 48h after treated by cycloxaprid LC10 concentration (HYFA) and 5 no-treatment female adults (CKFA) via the Illumina sequencing. Three replicates were prepared. We then constructed a mass gene database that contains a total of 157,906,456 nucleotides (nt). After eliminating low quality reads from the raw reads, there were 45,934,376 average clean reads remained in cycloxaprid-treatment, which accounted for 96.00% of the raw reads and 42,495,937 average clean reads remained in no-treatment, which accounted for 93.90% of their raw reads. The short reads assembly software Trinity was used to assemble, compare and ligate all clean data so as to get the unigenes from the *S. furcifera* transcriptome. These reads were assembled into 131,601 unigenes, the mean length is 720 bp. The 67.38% of unigenes ranged from 200-500 bp and 15.28% of unigenes ranged from 500-1000 bp. The 17.33% of unigenes ranged from more than 1000 bp in length. (Figure 1,Table 2, 3)

Unigenes functional annotation by NR, GO, KOG and KEGG

The results of unigenes functional annotation by non-redundant protein sequences (NR), Gene Ontology (GO), euKaryotic Ortholog Groups (KOG) and kyoto Encyclopedia of Genes and Genomes (KEGG), etc were showed in Table 4.

A total of 26,204 unigenes returned an above cut-off blast hit to NR database of the National Center for Biotechnology (NCBI), which account to 19.91% of 131,601 unigenes from the *S. furcifera* transcriptome. The species distribution of the top blastx hits for each unique sequence was shown in Figure 2. The unambiguous assembled sequences revealed that the percentage of matches sequences was 15.3% from *zootermopsis nevadensis*, followed by *Acyrthosiphon pisum* (5.2%), *Tribolium castaneum* (4.7%), and the greatest number of no-matches was 67.8%.

Based on the *S. furcifera* transcriptome assembly, 27,079 (20.57%) sequences were annotated in the GO database, which were divided into a total of 49 groups in three ontology categories (biological process, cellular component, molecular function). The "biological process" ontology category contains 24 groups. The largest group is "cellular process" with 15,163 unigenes, followed by "metabolic process" with 14,033 unigenes and the smallest group is "rhythmic process" with 30 unigenes. The "cellular component" ontology category contains 17 groups. The largest groups are "cell" and "cell part" with the same numbers, 8,460 unigenes, respectively. And the smallest group is "extracellular matrix component" with only four unigenes. The "molecular function" ontology category contains 8 groups. The largest group is "binding" with 14,136 unigenes, and the smallest group is "transcription factor activity, protein binding" with 314 unigenes (Figure 3).

In order to annotate the detail function of genes, KOG database was used. In total, 15,447 unigenes (11.76%) were annotated and these genes were divided into 26 categories. and a total of 3,104 unigenes (20.06%) were placed into the "General function prediction only" category. Followed by "signal transduction mechanisms" (1,986, 12.83%), "posttranslation modification, protein turnover, chaperones" (1,585, 10.24%), and the smallest category is "Cell motility" with 21 unigenes. There are three unigenes annotated "Unamed protein", accounting for only 0.02% of the functionally annotated unigenes.(Figure 4)

To identify the genes that involved in metabolic pathways, a total of 15,058 unigenes (11.44%) were mapped to the KEGG pathway database. These unigenes were divided into 32 pathways. The largest pathway is "signal transduction" with 1,829 unigenes (15.98%). Followed by "endocrine system" with 1,089 unigenes (9.52%). The smallest pathway is "Biosynthesis of other secondary metabolites" with

only 33 unigenes (0.2%).(Figure 5).

To validate the transcriptome, we compared the expression profiles of the cycloxaprid-treatment and no-treatment using qRT-PCR. We selected 18 unigenes randomly, Primers used in qRT-PCR for validation of DEGs were shown in Table 5. 14 of which demonstrated a concordant direction of change and 4 unigenes were inconsistent between transcriptome and qRT-PCR (Table 6). The results indicated that our results are reliable.

SSRs detection analysis

By using *S. furcifera* transcriptome sequences, we have identified 15,868 SSRs. According to predictions, about 10.56% of protein-coding sequences possessed such repeats, of which 45.1% were trinucleotide repeats, with (AAG/CTT)n being the most frequent motif (43.13%), followed by 36.82% mononucleotide, 16.49% dinucleotide, 1.42% tetranucleotide, 0.107% pentanucleotide, 0.063% hexanucleotide repeats (Figure 6). These results are confirmed with these reports showing that trinucleotide repeats (AAG/CTT)n are the most generous microsatellites in coding ESTs [16][29][30]. Similarly, Xu(2012) also reported that (AAG)n was the most frequent motif (39.8%) in *S. furcifera*[16]. However, it had been confirmed by the former work that (AAC)n is the most frequent motif in the small brown planthopper (*L. striatellus*)[26]. The difference of predicted trinucleotide repeats in the two transcriptomes may give an expression on distinct adaptability of these insects. The numerous potential molecular markers gained from our study would play a particularly important role on genetic mapping, genotyping and parentage analysis of these insect species[16].

Identification of DEGs

To identify DEGs, the numbers of clean reads for each gene were counted, then individual sets of reads were mapped back to the previously assembled transcript and counted as a proxy for gene expression. DESeq[31] was used to identify the differentially expressed transcripts between the two samples (HYFA and CKFA), padj<0.05 (with biological replicates). The numbers of up-regulated genes was 131 and down-regulated was 228, in total 359 DEGs (Figure 7).

217 DEGs were annotated in the GO database, inluding 65 up-regulated genes, which was approximatelty half of the 131 genes, and 152 down-regulated, which accounted to 66.67% of the

228 genes. These DEGs were assigned into groups according to these genes functions, biological process, cellular component and molecular function, and in the biological process, the largest groups were annotated into the "phosphorylation" and "protein phosphorylation". In the "cellular component" ontology category, the main groups are showed in Figure 8. The largest group of the "molecular function" ontology category is "catalytic activity".

The DEGs were mapped to the KEGG pathway database, which involved in metabolic pathways, These genes were divided into 179 pathways. The largest pathway is "Pathways in cancer" with 59 unigenes. Followed by "Proteoglycans in cancer" with 40 unigenes. "Rap1 signaling pathway" with 38 genes and "Prostate cancer" with 37 genes (Figure 9).

Identification of unigenes and DEGs related to insecticide-resistance

In all unigenes of the *S. furcifera* transcriptome, by these keywords related with insecticide resistance, we could found some metabolic resistance genes, 237 cytochrome P450 genes, 48 glutathione -s transferases (GSTs), 19 carboxylesterases (CarE), and target-site resistance genes, including 17 nicotinic acetylcholine receptor (nAChRs), 37 γ-aminobutyric acid receptors (GABA receptors), 50 acetylcholinesterase (AchEs), 99 sodium channels, etc (Table 7).

Based on the annotation results of basedata, we identified 3 categories of genes that associated with metabolic resistance, 2 Cytochrome P450s and 2 GSTs and target-site resistance, 1 Cadherin, etc in 359 DEGs.(Table 8)

Conclusion

In this study, we obtained 131,601 unigenes with mean lengths of 720 bp, which are a major genomic resource for clarifying the genes expression induced by cycloxaprid in *S. furcifera*. A total of 29,326 unigenes matched known proteins in the NCBI database. A total 15,868 simple sequence repeats were identified. 359 differentially expressed genes could be related with the cycloxaprid-induction in *S. furcifera*. And 22 unigenes may associated with cycloxaprid. such as cytochrome P450, GST, ACP, cadherin, etc.

Discussion

Insect resistance to insecticides generally related to each of three main mechanisms: lower epidermal

tissue penetration; metabolic resistance[32], which include the over-expression or detoxification of some critical enzymes, such as cytochrome P450[4][33][34], esterase and GST[35]. And target-site resistance, such as GABA receptor[36], AChE, sodium channel[37] etc.

Cytochrome P450 superfamilies were universal and constituted by many complex members, including 70 families, with 127 subfamilies, of hydrophobic, hemecontaining enzymes. P450s are involved in the biosynthesis of several essential endogenous compounds and the detoxification of many xenobiotics[38][39]. In insects, P450s are induced by a wide range of inducers[40] and could mediate resistance to all classes of insecticides[41]. And overexpression of P450 genes has been reported to be related with development of insecticide resistance in many insect species, such as *Nilaparvata lugens* (Stål) to imidaclorprid[33][42], *Laodelphax striatellus* (Fallen) to ethiprole[43], *Musca domestica* to phenobarbital[39], *Anopheles gambiae*[44][45] and *Meligethes aeneus* Fabricius[35] to pyrethroids, etc. More than half of the P450 genes belong to either the CYP4 or CYP6 families[46][47]. The CYP4 family includes sequences from vertebrates and are involved in hydroxylations of steroids, fatty acids and prostaglandins[48], whereas CYP6 enzymes, insect specific, appear to be involved in the metabolism of xenobiotics and plant products, e.g., pyrethroids and furanocoumarins[49]. In our study, only 2 P450 genes from DEGs were annotated as 1 CYP 4 family and 1 CYP 6 family.

Glutathione -s transferases (GSTs) contain many multifunctional detoxification enzymes and are indispensable in the process of insecticides metabolism, especially organophosphorus insecticides[50]. GSTs could catalyzed the conjugation of reduced glutathione (GSH) with electrophilic endogenous and xenobiotic compounds and end products of lipid peroxidation[51]. The increased expression and activity of GSTs has been clarified as one of mechanisms of insect resistance[52][53]. GSTs have been known to confer resistance to organochlorine, organophosphorous and pyrethroid, such as mosquitoes to DDT[54][55], *Musca domestic*a L. to diazinon, parathion and diazoxon [56], *Plutella xylostella* to methyl parathion[57], *Spodoptera litura* to Chlorpyrifos[58], *Nilaparvata lugens* to permethrin and lambda-cyhalothrin[59][60], *Nilaparvata lugens* to pyrethroid[60], *Bombyx mori* to fenpropathrin[61], *Leptinotarsa decemlineata* to cyhalothrin, fipronil and endosulfan [62], etc. In the study, 48 unigenes in the *S. furcifera* transcripome belong to GSTs , but only two DEGs code

glutathione -s transferase were identified.

Phosphatases, including acid and alkaline phosphatases(ACP and ALP), are capable of hydrolysis and transphosphorylation. They are important in the metabolism of carbohydrates, phospholipids and nucleotides and have been reported as enzymes significant in resistance to insecticides. ACP belongs to a group of enzymes that hydrolyze phosphomonoesters at acidic pH[63] and occupies a significant position in the detoxification of toxic compounds entering the body, acts as an enzymatic defence against foreign compounds and plays an essential role in maintaining normal physiological functions[64-68]. In the study, only one gene was founded to code acid phosphatases in 359 DEGs.

Cadherins represent a large and complicated family of calcium-dependent, transmembrane glycoproteins and a cytoplasmic tail that binds catenins which support to link the cadherin to the actin cytoskeleton, as well as also function in cellular signaling[69-72] and are responsible for maintaining the integrity of cell-cell contacts in multicellular organisms. In addition, cadherins include a class of proteins which were known to be related with Bt Cry protein binding and toxicity to Lepidoptera, Diptera and Coleoptera insects[73][74], and have been identified in other invertebrates as crucial target receptors for Cry toxins[75-77]. *Plutella xylostella* was the first insect species which reported to evolve resistance to Bt in field populations[78] and its high resistance levels to Bt have been confirmed with the loss of binding affinity of Cry toxins to their protein receptors, including cadherin, on the midgut brush border membrane[79][80]. Vadlamudi et al reported that a cadherin localized in the midgut epithelium of *Manduca sexta* serves as the receptor for Cry1A toxins of *Bacillus thuringiensis*[81][82]. Gahan et al.(2001) showed that the resistance to Cry1Ac toxin in *Heliothis virescens* is associated to retrotransposon-mediated disruption of a specific cadherin gene. Moreover, confirmed that midgut epithelial cadherins participated directly in the entomopathogenicity of *B. thuringiensis*[76]. However, there is little evidence that cadherin was related with the resistance to other insecticides beside Cry toxins. In our study, only one gene from DEGs could code cadherin.

Methods

Insects and Insecticides

S. furcifera were collected from Huaxi district of Guiyang in Guizhou Province, China in July 2014 and

bred down 20 generations under condition as hereinafter. The insects were reared on 10 day old rice seedlings cultured in plastic boxes (34×23.5×20cm) at 25±1°C temperature, 50% to 60% relative humidity, and L16:D8 h photoperiod in constant-temperature incubator[83].

Technical grade: cycloxaprid (97%) was provided by Shengnong pesticide Co., Ltd. Shanghai, China.

Bioassay

The rice-stem dipping method [22][84][85] was used for the dose-responses of *S. furcifera* to cycloxaprid. Cycloxaprid was firstly dissolved into mother liquid by acetonitrile plus 10% Triton-100 (m/V; Solarbio Science & Technology Co., Ltd. Beijing, China) as an emulsifer. Then the mother liquid were serially diluted into 5-9 different concentrations with distilled water, respectively. The mortality was recorded 96h later for cycloxaprid. The nymphs were considered dead if they failed to move when gently prodded with a fine bristle. The mortality data were corrected by the control mortality using Abbott's formula. Regression equation (LC-P line)、LC50 values (mg■L-1)、10% lethal concentration(LC10) and 95% confidence interval (CI) etc were calculated by using Data Processing System (DPS ver. 8.05) software (Hangzhou RuiFeng Information Technology CO., LTD. Hangzhou, China)[83][86].

Sample collection and preparation

The mother liquid of cycloxaprid was diluted into 1×LC10 concentration and distilled water was used as the control. The rice seedlings were dipped into these solution for 30s, respectively. More than one hundred of the fifth instar nymphs were introduced on these treated rice seedlings when air-dried at room temperature. After 48h treated, emergency adults would be distinguished between male and female and collected in EP tubes, respectively. Then, 48h, 5 female adults (HYFA) were randomly sellected for subsequent RNA isolation, transcriptome sequencing. Three replicates were prepared (plus the control (no-treatment) (CKFA) in total 30 nymphs). All collected bodies were immediately frozen by liquid nitrogen and stored at -80°C until use[83].

RNA isolation, Library construction

Qubit® RNA Assay Kit (Life Technologies, CA, USA) was used to measure RNA concentration. 1% agarose gels was used to monitor RNA degradation and contamination. The NanoPhotometer®

spectrophotometer (IMPLEN, CA, USA) was used to check the RNA purity. The RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA) was used to assess RNA integrity.

Transcriptome sequencing of *S. furcifera* was finished by Beijing Novogene Bioinformatics Technology Co., Ltd (Beijing, China).

1.5 μg total RNA for each sample was used for the RNA sample preparations. NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) was used to generate sequencing libraries. OligoT attached magnetic beads was used to enrich mRNA from total RNA. Then, mRNA was interrupted into short fragments via adding fragmentation Buffer (5X). Random hexamer primer and M-MuLV Reverse Transcriptase (RNase H-) was used to synthesize the first strand cDNA. Subsequently, DNA Polymerase I, RNase H, dNTPs and Buffer were used to synthesize the second strand cDNA. Polymerase could converted the remaining overhangs into blunt ends. The double strand cDNA were purified with AMPure XP beads. After adenylation of 3’ ends of DNA fragments, NEBNext Adaptor with hairpin loop structure were ligated to prepare for hybridization. After terminal repaired and plus A-tail and sequencing connector, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA) so as to select cDNA fragments of 150-200 bp in length preferentially. Subsequently polymerase chain reaction (PCR) was performed with T100 thermal cycler PCR Instrument (Bio-rad, USA) and then PCR products were purified with AMPure XP system. Finally, library quality (insert size) was assessed on the Agilent 2100 system and effective concentration of the library was analysized with Q-PCR (>2nM). Illumina Hiseq platform was used to sequence the library and generate the paired-end clean reads. TruSeq PE Cluster Kit v3-cBot-HS (Illumia) was used to perform the clustering of the index-coded samples on a cBot Cluster Generation System.

Two transcriptomes of *S. furcifera* were constructed treatment by cycloxaprid (HYFA) and no-treatment (CKFA)[83].

Data analysis

Quality control

Raw data (raw reads) of fastq format were firstly processed through in-house perl scripts. In this step,

excluding reads containing adapter, ploy-N and low quality reads from raw data, clean reads with high quality were obtained so as to subsequent analysis, Q20, Q30, GC-content and sequence duplication level of the clean reads calculated. All the downstream analyses were based on clean data with high quality[83].

Transcriptome assembly

The left files (read1 files) from all libraries/samples were pooled into one big left.fq file, and right files (read2 files) into one big right.fq file. Transcriptome assembly was accomplished based on the left.fq and right.fq using Trinity[87] with min_kmer_cov set to 2 by default and all other parameters set default.

Gene function was annotated based on the following databases: NR (Non-Redundant protein sequences)；NT (Non-Redundant nucleotide sequences)；Pfam (Protein family)；KOG (euKaryotic of Orthologous Groups of proteins)；Swiss-Prot (A manually annotated and reviewed protein sequence database)；KEGG (Kyoto Encyclopedia of Genes and Genomes)；GO (Gene Ontology)[83].

SSR detection and primer design

SSR of the transcriptome were identified using MISA (http://pgrc.ipk-gatersleben.de/misa/misa.html), and primer for each SSR was designed using Primer 5.

Differential expression analysis

RSEM were used to estimate gene expression levels[88] for each sample. For the samples with biological replicates, the DESeq R package (1.10.1) was used to perform the differential expression analysis of two groups (HYFA and CKFA). The Benjamini and Hochberg’s approach was used to modify the resulting P values for controlling the false discovery rate. Genes with an adjusted P-value <0.05 was set as the threshold for obviously differential expression.

To verify the accuracy of the transcriptome, the expression levels of 18 genes were conducted by qRT-PCR. Total RNAs from each sample were extracted using Triozol RNA (Life Technology) and treated with DNase I (Invitrogen) according to the manufacturer’s protocol. The concentration of each RNA sample was adjusted to 1 mg/mL with nuclease-free water and total RNA was reverse transcribed in a 20ul reaction system using the BestarTM qPCR RT Kit (DBI). qRT-PCR was carried out on the

Bestar® SybrGreen qPCR mastermix (DBI, Germany) under the following conditions: 95°C for 2 min; and 45 cycles of 95°C for 10 s, 60°C for 34 s, and 72°C for 30 s, followed by melting curve generation (60°C to 98 °C). Each gene was analyzed in triplicates, after which the average threshold cycle (CT) was calculated per sample, and an endogenous 18SrRNA gene (GenBank accession number: AB625607) of *S. furcifera* was used for internal normalization. A control sample without template was used to monitor contamination and determine the degree of dimer formation in the process of experiment[83].

Abbreviation

SRBSDV	The southern rice black-streaked dwarf virus
S. furcifera	*Sogatella furcifera* (Horváth)
CDS	Coding DNA Sequence
SSRs	Simple Sequence Repeats
DEGs	differentially expressed genes
GST	Glutathione-s transferase
ACP	Acid phosphatase
nAChRs	nicotinic acetylcholine receptors
CarE	Carboxylesterases
GABA receptors	γ-aminobutyric acid receptors
AchEs	Acetylcholinesterase
ACP	Acid Phosphatase
DNA	deoxyribonucleic acid
CYP4	Cytochrome P450 4 family
CYP6	Cytochrome P450 6 family
LC10/50	Lethal Concentration
CI	Confidence Interval
OD	optical density
RIN	RNA integrity number
RNA	ribonucleic acid
PCR	polymerase chain reaction
cDNA	complementary DNA
bp	base pairs
nt	nucleotides
dNTP	deoxyribonucleoside triphosphate
NR	non-redundant protein sequences
NT	nucleotide sequences
Pfam	protein family
KOG	euKaryotic Ortholog Groups
KEGG	kyoto Encyclopedia of Genes and Genomes
GO	Gene Ontology
FPKM	expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced
mRNA	messenger ribonucleic acid
NCBI	national center for biotechnology
qRT-PCR	Quantitative Real-time PCR
ESTs	Expressed Sequence tags
M	molarity
h	hour
min	minute
mg	milligram
ml	milliliter
μl	microliter
sec	second
EP	eppendorf tube
g	gravity
16L:8D	16 Light:8 Dark
FL	Fiducial limits
USA	United States of American
CA	California
CT	The average threshold cycle

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data Availability Statement: All data generated or analysed during this study are included in this published article.

The data that support the findings of this study are available from NCBI SRA database(Accession number: SRR4294200 and SRR4294203), but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of NCBI SRA database.

Competing Interests

This manuscript and its authors are not involved in any potential conflicts of interest, including financial interests and relationships and affiliations.

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Authors' contributions

Mrs. Jin JX analyzed and verified the transcriptome data of *S. furcifera* inducing by cycloxaprid and writed this manuscript. Mr. Ye ZC and Mrs. Cheng Y gave assistance in processing experimental data. Mrs. Li WH and Mr. Zhou YH provided help in rearing *S. furcifera* in our laboratory. Mr. Li FL and Jin DC were major contributors in amending this manuscript. All authors read and approved the final manuscript.

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Tables

Table 1 The RNA quality evaluation of *S. furcifera* samples treated with cycloxaprid (HYFA) and the untreated (CKFA)

Sample)	Concentration (ng/μl)	Volume (μl)	Quality) (μg)	OD260/280	RIN value
HYFA1	770	39	30.03	2.005	7.3
HYFA2	536	39	20.9	1.971	7.2
HYFA3	514	38	19.53	1.962	8.8
CKFA1	826	38	31.39	1.986	7.2
CKFA2	688	38	26.14	2	7.2
CKFA3	640	38	24.32	1.976	7.1

Table 2 The quality analysis of transcriptome data

Sample	Raw Reads	Clean Reads	Clean Bases	Error(%)	Q20(%)	Q30(%)	GC Content(%)
HYFA1	48378522	46420430	6.96G	0.02	96.24	91.5	36.53
HYFA2	47283654	45483546	6.82G	0.02	96.42	91.69	34.89
HYFA3	47880270	45899152	6.88G	0.02	96.23	91.44	38.66
CKFA1	45184564	42395408	6.36G	0.02	96.62	91.98	36.04
CKFA2	45098544	42261462	6.34G	0.02	96.44	91.6	35.35
CKFA3	45484342	42830940	6.42G	0.02	96.68	92.05	35.66

Table 3 The data analysis of transcripts and unigenes in *S. furcifera* transcriptome treated with cycloxaprid

	transcripts				Unigenes			
length interval	200-500bp	500-1kbp	1k-2kbp	>2kbp	200-500bp	500-1kbp	1k-2kbp	>2kbp
	102129	29696	21527	21498	88679	20109	11915	10898
Total Number	174850				131601			
mean length	903				720			
N50	1895				1412			
N90	308				263			
total nucleotides	157906456				94739951			

Table 4 Unigenes functional annotation by Nr, GO, KOG and KEGG, etc

	Number of Unigenes	Percentage (%)
Annotated in NR	26204	19.91
Annotated in NT	15128	11.49
Annotated in KO	11445	8.69
Annotated in SwissProt	23028	17.49
Annotated in PFAM	26899	20.43
Annotated in GO	27079	20.57
Annotated in KOG	15477	11.76
Annotated in all Databases	4793	3.64
Annotated in at least one Database	38534	29.28
Total Unigenes	131601	100

Table 5 The primers for valication to the transcriptome treated by cycloxaprid

Name		Sequence	Product size（bp）
18SrRNA Reference genes	Forward：	GCCCCGTAATCGGAATGAGT	205
	Reverse：	GACAAGACGTCCCGCAAAAC	
c74469_g1	Forward：	CTACTCGGCCTACCCGTACT	157
	Reverse：	CGAAGCTATTGACGGTCGGA	
c76759_g1	Forward：	TGTATGAACGTCGCAAGGCT	96

	Reverse	AGCGCCTATATTCTTCCCGC	
c68340_g1	Forward	GCGAATTGGTCATACCACGC	143
	Reverse	AGCCTGACGTCAATCAAGGG	
c50811_g1	Forward	CCGTCGTCTTCTTCCTGTAG	68
	Reverse	CGTCTCCTTCTTCGTGTTCC	
c72913_g3	Forward	CTGGTGTTATTAGTGACGGTTGTAG	228
	Reverse	TAGGAGTGCTGGCGAGAGG	
c82227_g1	Forward	AAGGAGGAGCAGGCAGAG	225
	Reverse	AGTGGCGAATGTTGGAAGG	
c72647_g3	Forward	GCCTTCCACCTACCGACATC	247
	Reverse	TCCTCTCACAACTGCCATCATC	
c82813_g1	Forward	CTTCTCCGAGGCATCAATC	54
	Reverse	GGACTCAGATAGCACATACG	
c72170_g1	Forward	CCATATTCTCAATGCTGCCTCTCAAG	232
	Reverse	TCATCCGTATCCATCGCCATCATC	
c83438_g1	Forward	ATCCTTGGTGTTGGCTACGG	100
	Reverse	TGTATATTGCGCAGGGCCAA	
c79766_g1	Forward	CTAGCAGTCTACGCCGAAGG	77
	Reverse	AAGCCCATGCCTTTGCCTAT	
c79304_g1	Forward	GTAGGCGAGTACGTTAGGCG	118

	Reverse	GACCCCATCCTGAGCCAAAT	
c76099_g1	Forward	GGCTGCTGTGGAAATGGTTG	184
	Reverse	AGCATCAAAGGCAAGCAAGC	
c70633_g2	Forward	CTCAGTGCCATGCCACCATA	107
	Reverse	CCCATGTATCGCAACCCTGT	
c67652_g2	Forward	TGATGCGGAGAAGGTATCGC	172
	Reverse	CTCACCCTCTCGGACTGGTA	
c62218_g1	Forward	CATGTGCAAGGCATTGTGCT	155
	Reverse	AGCTGCGTATTCAGTGCCAT	
c45748_g1	Forward	AGGAAGCCGTACCAAATCCA	231
	Reverse	GGGGTAGCACTACACCCAGT	
c43029_g1	Forward	AAGGGAAGGAAGAGCATGGC	159
	Reverse	TATCATCACCGTCATGGGCG	

Table 6 The validation to the transcriptsome of *S. furcifera* treated by cycloxaprid in qRT-PCR

Gene ID	Transcriptsome			qRT-PCR			Consistency
	XDFA_readcount	CKFA_readcount	log2Fold Change	The mean Ct of XDFA	The mean Ct of CKFA	F-value (2-△△Ct)	
C43029_g1	76.02	166.78	-1.13	17.79	16.14	0.32	Yes
C45748_g1	126.47	10.22	3.63	17.82	17.60	0.86	No
C50811_g1	0.00	9.67	-	24.28	24.50	1.16	No
C67652_g2	68.47	206.99	-1.60	18.61	17.03	0.34	Yes
C68340_g1	2002.36	0.00	-	8.92	25.91	130384.02	Yes
C70633_g2	0.98	102.63	-6.70	23.73	18.95	0.04	Yes
C72170_g1	8.88	102.26	-3.53	20.40	16.49	0.07	Yes
c74469_g1	1407.52	3557.59	-1.34	10.89	9.91	0.50	Yes
C72647_g3	456.84	160.97	1.50	14.91	15.33	1.35	Yes
C72913_g3	4289.74	2097.39	4.61	10.99	11.82	1.77	Yes
c76099_g1	0.00	118.31	-	22.41	22.06	0.78	Yes
C79304_g1	457.23	156.39	1.55	11.97	11.90	0.95	No
C79766_g1	243.06	114.95	1.08	16.07	16.57	1.41	Yes
C82227_g1	0.00	40.41	-	23.02	22.79	0.85	Yes
C83438_g1	0.00	119.94	-	24.12	24.67	1.47	No
C76759_g1	30.80	2057.69	-6.06	11.79	10.39	0.38	Yes
C62218_g1	1.99	517.62	-8.02	20.65	11.57	0.00	Yes
C82813_g1	0.00	43.43	-	16.21	15.19	0.49	Yes

Table 7 The statistic information for special unigenes associated with insecticide resistance

Resistance-related gene Category		Unigenes Number	Maximum Unigenes length	Minimum Unigenes length	Average length
Metabolic Resistance	Cytochrome P450	237	7778	201	1261
	Glutathione -s transferase (GST)	48	2802	202	721
	Carboxylesterase (CarE)	19	6786	270	2803
	Acid phosphatase (ACP)	91	26413	207	3146
Target Resistance	Nicotinic Acetylcholine Receptor (nAChRs)	17	6489	222	1654
	γ-aminobutyric acid receptor (GABA receptor)	37	8961	211	2810
	sodium channel	99	9011	202	1243
	Acetylcholinesterase (AChE)	50	5143	209	1838
	ATP synthase	236	8813	201	1319
	Cytochrome b (Cyt b)	187	12444	201	1566
	Cadherin	76	12444	205	1905

Table 8 Identification of DEGs associated with insecticide resistance

Gene function	Gene length	pval
Cytochrome P450	1917	1.12E-17
	2381	1.74E-05
Glutathione S-transferase(GST)	1523	2.30E-09
	977	2.14E-05
Acid Phosphatase(ACP)	896	2.34E-08
Cadherin	502	2.74E-06

Figures

Figure 1

The length distribution of transcripts and unigenes of Sogatella furcifera. The x axis shows the lengths of transcripts and unigenes and the y axis shows the number of transcripts and unigenes.

Figure 2

Species distribution □E-value distribution and similarity distribution of unigene against the NCBI. A-Species distribution of unigene BLASTX results against the NCBI-NR protein database. B-E-value distribution of unigene against the NCBI-NR protein database. C-similarity istribution of unigene against the NCBI-NR protein database.

Figure 3

Comparison of GO classification of putative functions of genes from cycloxaprid treatment and no treatment of S. furcifera samples. The x axis shows subgroups of molecular functions from GO classification and the y axis shows the number of the matched unigene sequences.

Figure 4

The detail function of genes in KOG database. The x axis shows the groups name in KOG and the percentage of unigenes annotated in a group account to the total unigenes annotated in KOG database.

Figure 5

Comparison of KEGG pathway distributions. The x axis shows the percentage of unigenes annotated in a group account to the total unigenes annotated in KOG database.the y axis shows the name of KEGG pathway. The genes according to KEGG metabolic pathway involved was divided into five branches: A-Cellular Processes; B-Environmental Information Processing; C-Genetic Information Processing; D-Metabolism; E-Organismal Systems.

Figure 6

Distribution of simple sequence repeats motifs(SSR). The x axis shows the SSR repeat type the y axis shows the repeat counts. the z axis shows the number of SSR.

Figure 7

The volcanoplot of differentially expressed genes of S. Furcifera based on transcriptome.The red and green points represent genes up-regulated and down-regulated genes by the comparison between cycloxaprid treatment and no treatment of S. furcifera, respectively, the blue points represent genes that have no differences in regulation based on the criterion of FDR<0.05 and an absolute value of the log2(fold change) >1. HYFA represents the samples treated with cycloxaprid, LD10=0.5823mg·l-1 and CKFA represents the samples with no treatment.

Figure 8

Distribution of the significantly up- and down-regulated unigenes in the enriched terms of GO classification. The x axis shows the terms of GO classification, including (BP) Biological process. (MF) Molecular function. (CC) Cellular component.The y axis shows the number of genes eriched in a term. HYFA represents the samples treated with cycloxaprid, LC10=0.58mg·l-1 and CKFA represents the samples with no treatment.

Figure 9

KEGG pathway distributions of S. furcifera differentially expressed genes. A-KEGG pathway distributions of the down-regulated genes. B-KEGG pathway distributions of the up-regulated genes.