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Research article

Transcriptomic and proteomic analysis of resistance to matrine in *Oedaleus asiaticus* (Orthoptera: Oedipodidae)

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ABSTRACT

In order to determine the specific genes and proteins that are affected by matrine and play a role in regulating metabolism in the locust species *Oedaleus asiaticus*, we conducted RNA-seq, proteomic sequencing, and bioinformatics analyses on third-instar nymphs. These nymphs were divided into two groups: one group was grown under normal conditions, while the other group was treated with matrine. The purpose of this investigation was to gain insight into the molecular mechanisms underlying matrine resistance. Genes and proteins that exhibited differential expression were identified and subjected to analysis using bioinformatics software. The DESeq analysis revealed a total of 743 transcripts that were differentially expressed (DETs). Among these, 208 transcripts were up-regulated, and 535 were downregulated in ZO/ZCK. The iTRAQ discovered that 34 and 65 proteins were, respectively, up- and down-regulated in ZO/ZCK. Additionally, enrichment studies based on Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) were performed. The KEGG and GO analysis of the DEGs suggested the essential roles of matrine treatment in the regulation of *O. asiaticus*, especially via the biosynthesis of amino acids, glycolysis/gluconeogenesis, iInsulin signaling pathway and MAPK signaling pathway. The findings demonstrated that matrine exerted control of the growth of *O. asiaticus* via modulating the synthesis of metabolism and biosynthesis. Increased expression of detoxifying enzymes was observed, which may be related to matrine detoxification. These findings provide a basis for better comprehension of the molecular mechanism behind the regulation of development mediated by matrine in Asian locust hoppers.

1. Introduction

Locusts are a major agricultural pest throughout the world. Locust plagues occur almost every year, causing huge economic losses to agriculture and animal husbandry, as well as seriously affecting the development of natural ecosystems and the social economy [1,

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2]. *Oedaleus asiaticus* (Orthoptera:Acridoidea). *O. asiaticus* is a highly destructive locust found in the Northern Grasslands of China. It feeds mainly on about 12 types of grass, including Gramineae and Sedge, *Artemisia frigida*, *Leymus chinensis* (Trin.) Tzvel, *Cleistogenes squarrosa* (Trin.) Keng, and *Stipa capillata* L [3]. The grasslands of Inner Mongolia [4,5] are inhabited by this pest species, which is prevalent in Inner Mongolia, Gansu, Qinghai, and other northern agricultural and pastoral regions. Damage caused by this species can destroy over 90 % of the vegetation [6,7]. Although many strategies have been developed to control locust outbreaks, the use of insecticides is still currently the preferred measure [8]. Commonly used anti-locust insecticides include chemical, plant-based, and microbial agents [9]. Nevertheless, the prolonged and frequent application of a single insecticide can result in the emergence of different levels of resistance to a range of insecticides [10–12].

Sophora flavescens L. is a deciduous shrub that belongs to the Leguminosae family and has a large distribution in China. Matrine is a class of alkaloids extracted from *S. flavescens*, mainly quinazinones, and is considered one of the most successful and effective botanical insecticides [13,14]. It is a broad-spectrum insecticide with gastric and contact toxicity and works by paralyzing the central nervous system of the insect, resulting in protein solidification, clogging of stomata, and eventual suffocation [15]. The effects of matrine differ in different insects. Studies have found that matrine can be bidirectionally absorbed and transmitted to resist aphids on wheat and pepper [16]. Mortality after treatment with matrine has been reported to reach 66.9 %, 74.1 %, and 92.3 % on the third, seventh, and eleventh days, respectively, after treatment [17]. Matrine has the advantages of being highly efficient with low toxicity and causing no pollution or residue in the environment while promoting the growth of crops. Therefore, it aligns with the principles of environmentally friendly and sustainable practices in agricultural and animal husbandry. Currently, there are no studies related to insect resistance to matrine.

This study represents the initial exploration of the transcriptome and proteome of third-instar nymphs exposed to matrine. The objective was to evaluate the impact of this insecticide, understand the underlying mechanism behind matrine resistance, Elucidating the molecular mechanisms of *O. asiaticus* resistance to matrine, and establish a theoretical foundation for managing *O. asiaticus* and developing environmentally friendly control strategies. Generally speaking, this study will contribute to help us enrich the knowledge of matrine and will allow for a better understanding of their possible involvement in the regulation of gene expressions in *O. asiaticus*.

2. Study methodology

2.1. Insect rearing

Oedaleus asiaticus specimens were obtained from the Siziwang banner grassland in Inner Mongolia, China, on July 15, 2020. Normally developed locusts were raised in well-ventilated cages with a photoperiod of 14 h of light and 10 h of darkness and temperatures of 25 ± 2 °C during the day and 21 ± 2 °C at night. The relative humidity was maintained at 40 ± 5 %. Sixty in each cage were fed to the third-instar nymphs of *O. asiaticus* as a reserve for stable 2-3days. The locusts were fed with fresh, pesticide-free, and pest-free *Leymus chinensis* and wheat seedlings supplemented with a small amount of wheat bran daily in the morning and evening. The *Leymus chinensis* used in the experiment were all collected from Shaerqin base; the wheat variety was Changfeng 2112, and the seeds were purchased from Shaanxi Changfeng Seed Industry.

2.2. Induction treatment of matrine for O. asiaticus

The formulation consists of 98 % matrine cream, supplied by Beijing Qingyuanbao Biotechnology Co., LTD. The acetone was configured as the solvent to 5 % matrine EC (m/v) as the mother liquor, and 2.5 μL matrine with a sub-lethal dose of LD_{10} (0.021 μg/head) was applied to the abdominal segments of 60 third-instar nymphs using a micro syringe, allowing for three biological replicates. Acetone was applied to the same number of larvae as the control. The treated locusts were monitored in the insect-rearing room at 25 °C, and samples were collected 48 h after treatment. The locusts were submerged in liquid nitrogen and preserved at a temperature of −80 °C for subsequent study.

2.3. Transcriptome sequencing and gene annotation

For transcriptome sequencing, the samples were submitted to Biomarker Technologies Co, Ltd. (Beijing, China). The sequencing libraries were prepared utilizing the NEBNext®Ultra™ RNA Library Prep Kit for Illumina® (NEB, Ipswich, MA, USA), per the directions that the supplier provided. In order to specifically choose cDNA fragments that are 240 base pairs long, the library fragments were purified using the AMPure XP technology (Beckman Coulter, MA, USA). The evaluation of library quality was conducted using the Agilent Bioanalyzer 2100 (Santa Clara, CA, USA). The library preparations underwent sequencing using the Illumina Hiseq 6000 platform (San Diego, CA, USA). This process resulted in the production of paired-end reads. The raw data was processed to remove reads that contained adapters, poly-N, and low-quality reads, resulting in clean data. Simultaneously, the clean data was analyzed to determine sequence duplication levels, GC-content, Q30, and Q20. The RNA-Seq data was subjected to the novel assembly using the right.fq and left.fq files. Performing fq analysis using Trinity using the default options, except for setting the min_kmer_cov to 2 [17]. The assembled sequences were annotated using BLASTx and BLASTn with E-values $\leq 1 \times 10^{-5}$. The annotation was based on the following databases: Gene Ontology (GO), KEGG Ortholog database (KO), Pfam (Protein family), KOG/COG (Clusters of Orthologous Groups of proteins), Nt (NCBI non-redundant nucleotide sequences), Nr (NCBI non-redundant protein sequences), and Swiss-Prot (a manually annotated and reviewed protein sequence database).

The calculation of transcript expression levels was performed in conjunction with RNA-Seq using the Expectation-Maximization

(RSEM) method [18]. The expression levels of the transcripts were represented using the Fragments Per Kilobase of transcript per Million mapped reads (FPKM) metric. The DESeq2 package in R was utilized to determine differential expression [19]. To adjust p-values, the Benjamini and Hochberg techniques were utilized. FC (Fold change) $\geq$2 and FDR $<$0.05 genes were designated DEGs. Using the GOseq tool in R and the Wallenius non-central hyper-geometric distribution, GO enrichment analysis of DEGs was carried out [20]. KEGG database's enrichment of DEGs in pathways was examined using KOBAS software [21].

2.4. Protein extraction, TMT labeling, and LC-MS/MS analysis

To achieve a final volume of 300 μL, 50 μg of protein were diluted in 0.1 M Tris-HCl with 8 M urea. The ultrasonication of the solution lasted 1 s, was interrupted after 2 s, and continued for 120 s. Following 20 min at 4 °C centrifugation at 14 000 g, the protein content in the supernatant was determined, and SDS-PAGE was used to evaluate the protein quality. After thawing the tandem mass tag (TMT) reagent at room temperature, 41 μl of acetonitrile was added. This was followed by 5 min of shaking and a 20-min centrifugation at 14 000 g at 4 °C. A 100 μg enzyme-cut sample was mixed with the TMT reagent and left to react for 1 h at room temperature. The ammonia caused the process to stop. Following a vortex and centrifugation of the tagged samples, the residues were vacuum-freeze-dried.

The mobile phases for LC-MS/MS were produced as follows: solution B (80 % acetonitrile, 0.1 % formic acid) and solution A (100 % water, 0.1 % formic acid). Following lyophilization, the sample was reconstituted in 10 μL of solution A. It was then subjected to centrifugation at 14000*g* for 20 min at 4 °C. Subsequently, 1 μL of the resulting supernatant was used for analysis employing an ion source and a Q Exactive HF-X mass spectrometer (Thermo Fisher, USA). The temperature of the ion transfer tube was set to 275 °C, and the ion spray voltage was set to 2.4 kV. The mass spectrum was acquired using a data-dependent mode, and its entire scanning range was *m/z* 407–1500. The maximum C-trap injection duration was set to 20 ms, the AGC was set to 3×10^6, and the primary MS resolution was set to 60 000 (200 *m/z*). Secondary mass spectrometry (MS) was carried out after parent ions with the TOP 40 ionic strengths in the entire scan were selected for fragmentation by high-energy collision cracking (HCD). Peptide fragmentation and collision energy were set at 32 %, the maximum injection time was 86 ms, the AGC was 5×10^4, and the secondary MS resolution was 45 000 (200 *m/z*). Thus, the raw data (raw) was produced.

2.5. Real-time quantitative PCR

Eight DEGs associated with drug resistance were randomly selected from a transcriptome sequencing database for qRT-PCR verification. These included glutathione S-transferase (GST), cytochrome P450 (CYP450), mucin (MUC), heat shock protein (HSP), ATP-binding cassette transporter (ABC), cytochrome oxidase (CYT), glycerophosphodiester phosphodiesterase (GDE), and carbonyl reductase (NADPH). The gene-specific primers for O. asiaticus were designed using an online qRT-PCR primer design tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/; accessed on September 15, 2022), depending on the RNA-Seq information shown in Table 1. Employing an FTC-3000P Time Quantitative Thermal Cycler system (Funglyn Biotech, Richmond Hill, ON, Canada) and a BRYT Green® dye kit (Promega, Fitchburg, WI, USA), the qRT-PCR reaction was carried out. The reference gene, in this case, was β-actin. 20 μL of qRT-PCR master mix (Promega, USA), 7.2 μL of RNase-free H_2O, 0.4 μL of reverse primer (10 μmol/L), and 2 μL of cDNA, 0.4 μL of forward primer (10 μmol/L) were used in the qRT-PCR experiments. The amplification conditions for all reactions were as follows: a 10 min initial denaturation at 95 °C, 40 cycles of 15 s at 95 °C, 1 min at 60 °C, and 15 s at 72 °C. Using three biological and four technical replicates, the $2^{-\Delta\Delta Ct}$ technique [21] was utilized to examine the qRT-PCR data. The analysis of all datasets was done with SPSS v.20.0 (IBM SPSS Inc., Chicago, USA).

2.6. Statistical analysis

The analysis of all the data was done with GraphPad Prism (v.6.0). One-way ANOVA was used to examine differences in each target gene's expression levels following matrine treatments, and Tukey's Honestly Significant Difference (HSD) tests ($p < 0.05$) were then performed. All datasets were analyzed using SPSS 20.0 software. All data were presented as the mean ± SE (standard error).

Table 1
provides the primer data utilized in qRT-PCR analysis.

Gene	Forward primer	Reverse primer
OaALP	TTTGGTGCCACTGTGAATGT	CTCCACCAGTCATCTCAGCA
OaCYP450	CTGGAAACTTTGGCTGCTTC	AGAATGTTCTCCTCGCCAAA
OaCarE	ACCGTATTTTGCGAAGTTGG	CACCAGAGCCCGTCTTCTAC
OaAPN	GCAGCAACTTTAGGCTGTCC	GAGGCAACAAGACACCCATT
OaGST	CCCGTCATCCTCTATGGAAA	AATACGCCACAATTGCATGA
OaMUC	CAATCCCTCCACATCTGCTT	CCGGTCTTTGGATGTCAACT
OaABC	TGATTAGCTCCGCAAATTTCT	AACAGCCGCAGATGTTATCC
OaHSP	TCCTTGTTGGAGTCGAGCTT	ACTTTCGAGTTCCAGGCTGA
β-actin	CTACCACAGCCGAGCGAGAA	CCATCAGGCAGCTCGTAGGA

3. Results

3.1. An overview of the iTRAQ and RNA-seq data

Duplicate mRNA samples taken from the control and matrine-treated groups were used for RNA-seq analysis. The six samples yielded a transcriptome data set of 38.48 Gb. A total of 102 370 transcripts and 58 276 putative unigenes were found after mapping to the *O. asiaticus* cDNA database. The application of multidimensional scaling (MDS) demonstrated a significant level of consistency among biological replicates at each stage of development. In order to develop a deeper comprehension of the transcriptional patterns at different stages following matrine therapy, we conducted a comparative analysis between ZO (matrine treatment)and ZCK(control group) (ZO/ZCK). The DESeq analysis revealed a total of 743 transcripts that were differentially expressed (DETs). Among these, 208 transcripts were up-regulated, and 535 were downregulated in ZO/ZCK (Fig. 1), It is worth noting that both P450 and GST, which play crucial roles in insect detoxification processes, are upregulated. The significance threshold used was an FDR of <0.05 and an absolute value of log2ratio greater than 1 (Table S1). For the isobaric Tags for Relative and Absolute Quantification (iTRAQ)trials, the same comparisons (ZO/ZCK) were carried out. After evaluating the iTRAQ studies' reproducibility analysis, it was found that there was suitable reproducibility between three replicates in each development stage. The differentially expressed proteins (DEPs) were classified as proteins with a fold change of ≥ 1.2 and a P value < 0.05 in at least one replicate study, as well as the same change trend (ratio value greater than one for up-regulation and less than one for down-regulation) in the other replicate analyses. Consequently, it was discovered that 34 and 65 proteins were, respectively, up- and down-regulated in ZO/ZCK (Table S2).

3.2. Functional study of proteins and transcripts with variable expression

We used GO enrichment analysis to identify over-represented functional categories in DETs/DEPs compared to the entire transcriptome of *O. asiaticus* to acquire a better understanding of the possible functions linked to the alterations in the transcriptome and proteome. According to the GO enrichment analysis, 21 terms, 14 terms, and 19 were highly enriched in the molecular function, cellular component, and biological process categories at the transcriptome level for the ZO vs. ZCK comparison (Fig. 2A). For the ZO vs. ZCK comparison, GO analysis at the protein level revealed significant enrichment in 9, 9, and 8 items in the categories of molecular function, cellular component, and biological process (Fig. 2B). The 3 highest-ranking terms in the biological process category were single-organism process, cellular process, and metabolic process. In the cellular component category, the top 3 terms were cell part, cell, and membrane. Lastly, the molecular function category's top 3 terms consisted of transporter activity, binding, and catalytic activity. The findings demonstrated a considerable overlap in the enrichment of DEPs and DEGs between the transcriptome and proteomic data.

In order to examine the hypothetical molecular pathways responsible for the effects of matrine on *O. asiaticus*, the annotated DEGs and DEPs were analyzed using KEGG enrichment analysis. At the mRNA level, 87 DEGs were found to be enriched in 20 KEGG pathways, with the most significant enrichment seen in pathways associated with the biosynthesis of amino acids, glycolysis/gluconeogenesis, iInsulin signaling pathway, MAPK signaling pathway, arginine and proline metabolism, GnRH signaling pathway, aarbon metabolism, toxoplasmosis, choline metabolism in cancer, glutamatergic synapse, beta-alanine metabolism, ribosome, taurine and hypotaurine metabolism, influenza A, lysine biosynthesis, phospholipase D signaling pathway, leishmaniasis, pertussis, NF-κB signaling pathway, and glutamate, aspartate, and alanine, metabolism (Fig. 3 and Table 2).

At the protein level, a total of 49 DEPs were found to be enriched in 18 KEGG pathways. The most significant enrichment was observed in pathways related to the processing of protein in the endoplasmic reticulum (ER), lysosome, RNA transport, sucrose and starch metabolism, pentose and glucuronate interconversions, oxidative phosphorylation, ubiquitin-mediated proteolysis, drug metabolism - other enzymes, purine metabolism, proteasome, N-glycan biosynthesis, alanine, aspartate, and glutamate metabolism,

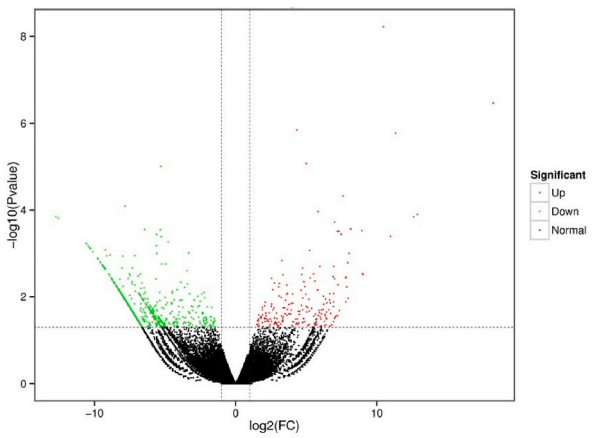

Fig. 1. Volcano plot of differentially expressed genes.

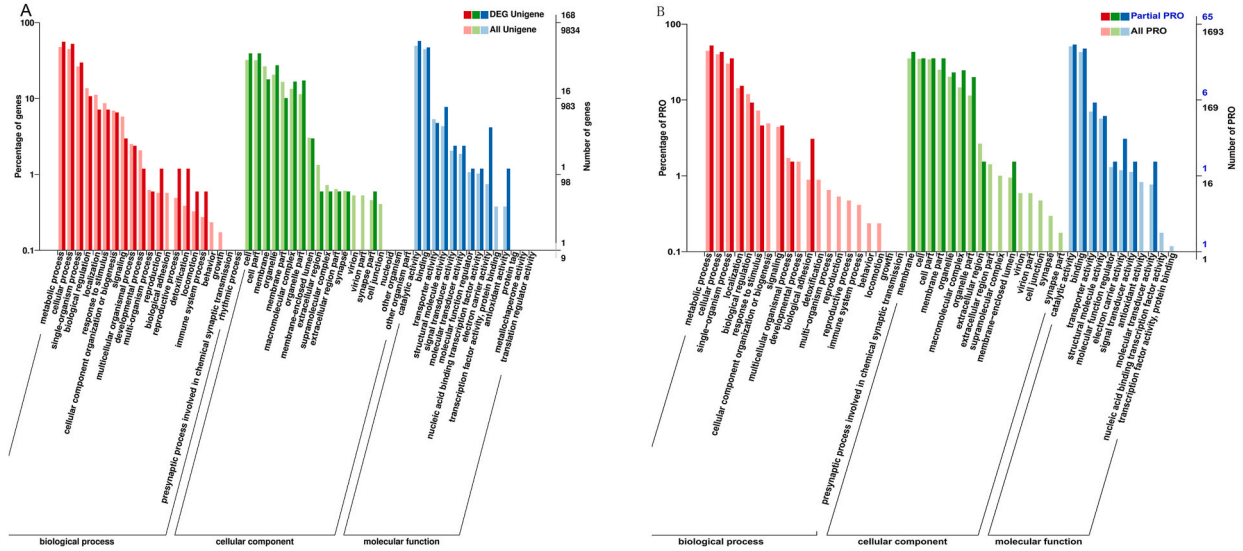

Fig. 2. Functional enrichment of proteins and transcripts with varying expression patterns. Compared to the entire genome's genes and all known proteins, the matrine-induced transcripts (**A**) and proteins (**B**) had an overrepresentation of GO enrichment (GO) groups. The distribution of proteins and transcripts that are differently expressed in the GO enrichment analysis is represented by dark green bars. Grey bars represent the distribution throughout the entire genome. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

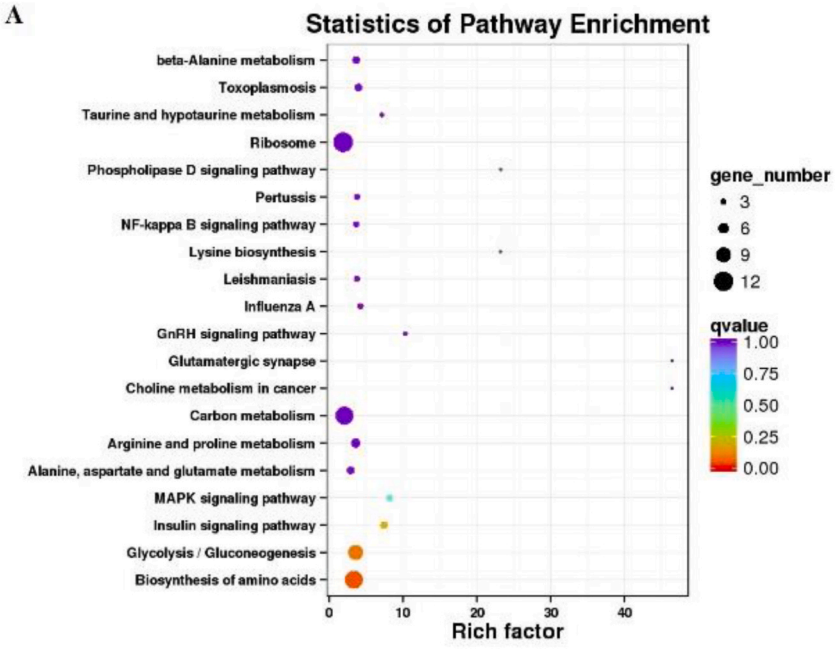

Fig. 3. KEGG pathways associated with significant enrichment of DEGs after matrine treatment.

phagosome, unsaturated fatty acids biosynthesis, fatty acid metabolism, mRNA surveillance pathway, spliceosome, and Carbon metabolism (Fig. 4 and Table 3).

3.3. Validation of DEGs by qRT-PCR

To confirm the results of Seq-RNA deep sequencing, 8 differentially expressed genes were randomly selected for qRT-PCR analysis (Fig. 5). The results showed that the expression patterns of 8 DEGs were similar to the results of Seq-RNA deep sequencing, which

Table 2
Enriched KEGG pathways in ZO vs. ZCK at the mRNA level.

Pathway	ko_ID	DEGs_in_Pathway	P-value	Corrected_P-value
Amino acids biosynthesis	ko01230	11	0.0004	0.0438
Glycolysis/Gluconeogenesis	ko00010	9	0.0008	0.0929
Insulin signaling pathway	ko04910	4	0.0018	0.1997
MAPK signaling pathway	ko04010	3	0.0053	0.5834
Arginine and proline metabolism	ko00330	5	0.0125	1
GnRH signaling pathway	ko04912	2	0.0150	1
Carbon metabolism	ko01200	11	0.0170	1
Toxoplasmosis	ko05145	4	0.0179	1
Choline metabolism in cancer	ko05231	1	0.0215	1
Glutamatergic synapse	ko04724	1	0.0215	1
beta-Alanine metabolism	ko00410	4	0.0234	1
Ribosome	ko03010	12	0.0244	1
Taurine and hypotaurine metabolism	ko00430	2	0.0307	1
Influenza A	ko05164	3	0.0332	1
Lysine biosynthesis	ko00300	1	0.0426	1
Phospholipase D signaling pathway	ko04072	1	0.0426	1
Leishmaniasis	ko05140	3	0.0445	1
Pertussis	ko05133	3	0.0445	1
NF-kappa B signaling pathway	ko04064	3	0.0476	1
Alanine, aspartate and glutamate metabolism	ko00250	4	0.0483	1

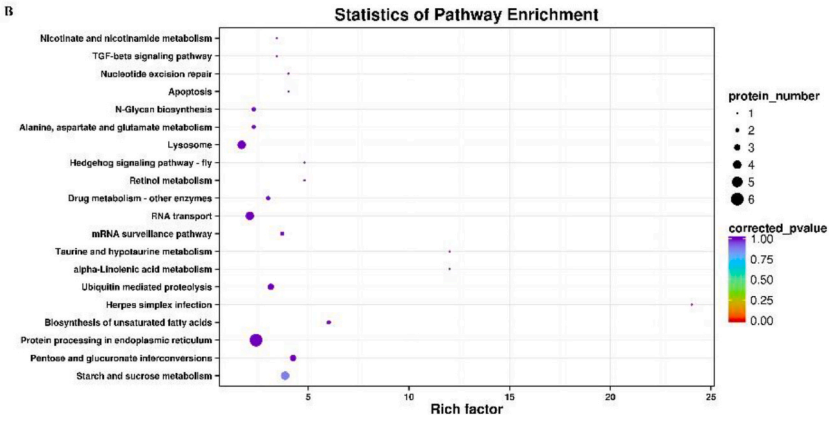

Fig. 4. shows the KEGG pathways that were enriched with DEPs following the administration of matrine.

Table 3
Enriched KEGG pathways in ZO vs. ZCK at the protein level.

Pathway	ko_ID	Diff_PRO_num
Protein processing in the ER	ko04141	6
Lysosome	ko04142	4
RNA transport	ko03013	4
Starch and sucrose metabolism	ko00500	4
Pentose and glucuronate interconversions	ko00040	3
Oxidative phosphorylation	ko00190	3
Ubiquitin mediated proteolysis	ko04120	3
Drug metabolism - other enzymes	ko00983	2
Purine metabolism	ko00230	2
Proteasome	ko03050	2
N-Glycan biosynthesis	ko00510	2
Glutamate, aspartate and alanine metabolism	ko00250	2
Phagosome	ko04145	2
Unsaturated fatty acids biosynthesis	ko01040	2
Fatty acid metabolism	ko01212	2
mRNA surveillance pathway	ko03015	2
Spliceosome	ko03040	2
Carbon metabolism	ko01200	2

proved the reliability of our Seq-RNA deep sequencing data.

3.4. Matrine treatment changes the phenotypic of *O. asiaticus*

In general, the body color of solitary individuals of *O. asiaticus* is green, which, upon exposure to matrine treatment changed to brown, including the color of legs and pronotum. The change in body color became more comprehensive with the longer duration of gregarious treatment (Fig. 6).

4. Discussion

O. asiaticus is a dominant pest in grasslands, resulting in serious harm to animal husbandry. After years of research by scientists, many methods have been devised for the control of *O. asiaticus* infestation, of which the most popular are insecticides b. Unfortunately, the excessive and inappropriate use of a single insecticide increases the likelihood of the insect developing resistance [22]. Thus, the study of drug resistance in insects has become a focus and hotspot of research. To date, while resistance-related genes have been identified in most insects, few have been reported in locusts. The extensive utilization of transcriptome sequencing in recent years has facilitated the identification of genes and regulatory networks linked to drug resistance in insects [23]. The HN strain of the mosquito, *Culex pipiens quinquefasciatus,* was first collected in the wild in 2013 when it was found to show significant resistance to deltamethrin. The mosquito has since been bred for generations in the laboratory without exposure to any pesticides and still shows resistance multiples above 4,000, suggesting that high levels of resistance to certain insecticides are difficult to reverse [24]. Therefore, it is critically important for green control to avoid the development of high insecticide resistance in locusts.

The mechanisms by which insects develop resistance to insecticides primarily include metabolic resistance, behavioral resistance, target-site resistance, and penetration resistance. Metabolic resistance is one of the most significant mechanisms through which insects develop resistance to insecticides. It mainly involves increasing the activity of detoxification enzymes, accelerating the metabolic rate of insecticides in insects, and thereby developing resistance to insecticides [25]. This process involves various enzymes and is a crucial mechanism of resistance. Insect detoxification enzymes belong to a heterogeneous enzyme system that can metabolize endogenous or exogenous substrates. Different enzymes play different roles in detoxifying and developing resistance to various insecticides. Currently, the focus of research is on several key enzyme systems: carboxylesterases (CarE), glutathione-S-transferases (GST), multifunctional oxidases (MFO), and cytochrome P450 (CYP450) [26].

GST is a vital enzyme involved in the detoxification process in insects and is vital in resistance to insecticides. Genes encoding GST enzymes are up-regulated or down-regulated by many substances in insects. Lumjuan et al. proposed that RNA interference silencing of certain GST genes increases the sensitivity of *Aedes aegypti* to pyrethroids and organophosphates, suggesting that these GST may also play a role in resistance to pyrethroid insecticides [27]. In this study, GST were up-regulated due to the stress of insecticides on locusts, thus activating a protective response to stress. A comparison of the activities of detoxifying enzymes in sensitive and resistant insect strains showed that the development of insecticide resistance was also associated with increased GST activity in insects *in vivo* [28,29].

P450s are defensive enzymes that have been discovered to be linked with resistance. Feyereisen et al. [30] demonstrated the involvement of P450 enzymes in the metabolism of multiple insecticides, and these enzymes are thus key to the development of insecticide resistance in insects. P450 enzymes can be divided into CYP2, CYP3, CYP4, and mitochondrial P450 clades according to their evolutionary relationships. Feyereisen et al. suggested that P450 enzymes play important roles in the adaptation of herbivorous insects to host plants, and, therefore, if an insect can detoxify insecticides applied to plants, it has the potential to become a destructive pest [31]. Many studies have shown that overexpression of cytochrome P450s, which are involved in pesticide detoxification, is an important cause of insect resistance to different types of insecticides [32]. Han proposed that the P450 gene expression levels showed a substantial rise following the administration of high quantities of Avermectin (179.66 μg/mL). This observation implies that P450 genes might have a crucial function in the detoxification and metabolism of Avermectin by Asian adult fruit moths [33]. The up-regulation of P450 transcription is associated with interactions between cis- and trans-acting regulating elements. These conclusions are consistent with the up-regulation of P450 expression in this study.

Zhang et al. conducted a study on plant cinnamyl alcohol dehydrogenase, which is present in both vascular and non-lignified tissues. They proposed that this enzyme may be involved in the plant's lignin defense pathway. The study found that the lignin content decreased by 93.4 % compared to wild-type plants, which aligns with the observed decrease in alcohol dehydrogenase expression [34,35].

5. Conclusion

During the present investigation, Illumina RNA-seq and proteomic sequencing were used to sequence and analyze 3rd-instar nymphs of *O. asiaticus* treated with matrine to identify the pathways underlying insecticide resistance to matrine in this insect. These pathways were found to be associated with metabolism, the processing of environmental information, and the interaction between the receptors of neuroactive ligands. The results establish a basis for a deeper comprehension of the molecular mechanism behind the growth regulation of *O. asiaticus* triggered by matrine., This study has only conducted preliminary omics analysis on the resistance of *O. asiaticus* to matrine. It is still some distance away from practical application. Further in-depth analysis of specific functional genes or proteins is needed, along with more functional studies, to help us clarify the regulatory mechanisms of *O. asiaticus* resistance to matrine. We hope to use insecticides more efficiently in practical *O. asiaticus* control efforts.

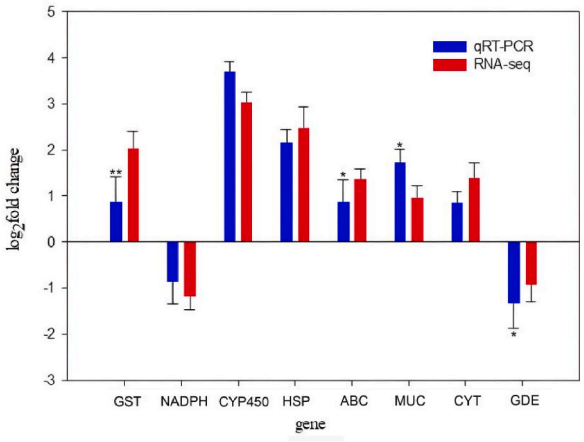

Fig. 5. Verification of deferentially expressed genes by qRT-PCR. The abscissa was the name of differentially expressed genes, and the ordinate was the expression value. *represents the significant difference at $P < 0.05$.

Fig. 6. Phenotypic change in *O. asiaticus* after matrine treatment.

Ethics approval and consent to participate

Not applicable.

Data availability statement

Data will be made available on request. Differential omics data have been deposited at Sequence Read Archive (SRA) (https://submit.ncbi.nlm.nih.gov/subs/sra/) with accession numbers PRJNA1126332.

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CRediT authorship contribution statement

Haibin Han: Writing – original draft, Validation, Methodology. **Hongyue Ma:** Writing – original draft, Data curation. **Xubing Yang:** Software, Data curation. **Jie Zhao:** Software, Methodology, Investigation. **Feng Yan:** Investigation. **Jingyi Zhang:** Data curation. **Shujing Gao:** Writing – review & editing, Project administration, Funding acquisition. **Yu Zhang:** Writing – review & editing, Software.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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