

Article

Metabolic and Behavioral Impacts of Gustatory Receptor *NIGr23* Silencing in the Brown Planthopper

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Abstract

The brown planthopper (BPH), *Nilaparvata lugens*, is the most destructive insect pest of rice. BPH infestations severely threaten rice yield worldwide. The gustatory receptor *NIGr23* plays a critical role in mediating the repulsive reaction to oxalic acid of the BPH. We integrated transcriptomic and proteomic analyses to determine the metabolic and behavioral consequences of *NIGr23* silencing. The RNAi-mediated knockdown of *NIGr23* increased body weight and honeydew production, indicating enhanced feeding activity. The results of multiomics profiling revealed disrupted lipid homeostasis, identifying 187 differentially expressed genes and 150 differentially expressed proteins. These genes were enriched in pathways including glycerophospholipid metabolism, fatty acid biosynthesis, and AMPK signaling. The results of biochemical assays showed that *NIGr23* silencing elevated triacylglycerol levels by 68.83%, and reduced glycerol and free fatty acid levels, suggesting impaired lipolysis. The *NIGr23* loss-of-function mutation mechanistically activates the AMPK pathway, suppresses lipid breakdown, and promotes energy storage. This study established *NIGr23* as a key regulator linking chemosensation to metabolic reprogramming, providing new insights into gustatory receptor-mediated energy homeostasis in the BPH.

Keywords: proteomics; transcriptomics; integrated methods; *Nilaparvata lugens*; rice pest; biocontrol



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1. Introduction

Rice is one of the most important staple crops in China, with over half of the population relying on rice as their primary food source [1]. The brown planthopper (BPH), *Nilaparvata lugens*, seriously threatens healthy rice plant growth, development, and reproduction [2]. Large BPH populations feed by piercing and sucking, which severely weakens plants and leads to considerable yield reductions. Additionally, BPHs act as vectors for various rice viruses, indirectly exacerbating crop damage. Therefore, effective measures for controlling this pest must be identified and implemented to safeguard rice production.

Gustatory receptors (GRs) are a family of seven transmembrane domain proteins that allow insects to appropriately respond to environmental cues [3,4]. Insects detect external chemical signals through GRs, which convert these signals into neural impulses and ultimately influence their behavioral patterns. This regulatory mechanism is crucial for insect survival and reproduction, affecting behaviors such as foraging, mate selection, oviposition site choice, and predator avoidance [5,6].

Advances in molecular biology and bioinformatics have led to the discovery of numerous GRs with sequenced genomes since the first insect GR was identified in *Drosophila melanogaster* [7]. A single receptor can respond to multiple ligands and a single ligand can activate multiple receptors in HEK293 cell lines and *Xenopus oocyte* expression systems. For example, *D. melanogaster* Gr32a and Gr33a detect strychnine [8] and nicotine [9], respectively, whereas Gr66a responds to strychnine, canavanine, and caffeine [10–12]. BmorGr53 and BmorGr16 detect caffeine and coumarin, whereas BmorGr18 responds to caffeine, pilocarpine, and coumarin in *Bombyx mori* [13]. Gr28 in *Pieris rapae* specifically recognizes sinigrin, which is a key stimulant in cruciferous plants [14]. These receptors influence feeding and ovipositioning through binding to specific ligands. However, downstream signaling mechanisms after ligand binding remain poorly understood.

Galactose binding in the BPH (*Nilaparvata lugens*) to NIGr11 activates the PI3K-AKT-PFK-ATP pathway via G proteins and insulin receptors, regulating ovipositioning [15]. In addition, the sugar receptor NIGr7 reduces vitellogenin (Vg) expression levels in female BPHs through interacting with ATP hydrolases [16]. Studies on GR signaling have primarily relied on calcium flux or electrophysiological recordings [9,17,18]; analyses of the downstream pathways have been limited [19].

Advances in RNA-seq and iTRAQ sequencing have enabled considerable progress in the multiomics analyses of insects. Examples include identifying the reproduction-related genes in the BPH [20], the Ca^{2+} signaling pathways linked to diapause in *Tetranychus urticae* [21], and the diapause mechanisms in *Aphidius gifuensis* [22]. Transcriptomic and proteomic analyses of *Myzus persicae* infected with cucumber mosaic virus were used to identify key regulators such as ribosomal proteins, cuticular proteins, and cytochrome P450 enzymes, the accumulation of which was altered after infection with the virus [23]. Integrated multiomics studies on *Leptinotarsa decemlineata* revealed 2840 proteins (409 upregulated and 200 downregulated) that were differentially expressed after exposure to cold proteins, highlighting their impact on selective metabolism and RNA-related processes [24]. These studies demonstrate how multiomics approaches have enhanced our understanding of post-transcriptional and translational regulation.

We employed RNA interference (RNAi) to silence the NIGr23 gene in the BPH and conducted integrated transcriptomic and proteomic analyses to compare differences in the mRNA and protein expression levels between NIGr23-knockdown and control groups. Our goal was to identify the molecular pathways linked to NIGr23 function, validate their roles, and establish a foundation for understanding GR-mediated behavioral regulation in the BPH. This study provides insights for guiding the development of biocontrol strategies for the BPH.

2. Materials and Methods

2.1. Insects

BPH populations were collected from Zunyi, Guizhou, China. The insects were maintained in the laboratory at 26 ± 2 °C under a 16:8 h (light/dark) photoperiod at a relative humidity level of 80% ($\pm 10\%$) on susceptible rice seedlings (Huang Huazhan variety).

2.2. Preparing and Injecting dsRNA into the BPH

Template DNA was prepared, dsRNA was synthesized as previously described [25], and experiments were performed in October 2023. Briefly, first-strand complementary DNA (cDNA) was synthesized using 1 µg of total RNA and a PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Kyoto, Japan). The full-length cDNA sequences of NIGr23 were amplified using 2× plus Taq HiFi PCR mix with blue dye (Mikx, Shenzhen, China), following the manufacturer's protocol. A T7 RiboMAX™ Express RNAi System (Promega,

Madison, WI, USA) was then used to synthesize dsRNA from the purified PCR products. All primers used for PCR are listed in Table S1. The ds*NIGr23* (239 bp) concentration was determined using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Approximately 250 ng of dsRNA was injected into newly eclosed female BPHs anesthetized with CO₂ for 20 s for RNAi experiments [25]. BPHs were reared on fresh rice plants after this injection. The gene silencing efficiency at different time points (24, 48, and 72 h after injection) was determined using quantitative reverse-transcription PCR (qRT-PCR).

2.3. RNA Extraction and qRT-PCR Analysis

The total RNA was prepared using a Magnetic Column RNA Extraction Kit (Mikx, Shenzhen, China) according to the instructions of the manufacturer. First-strand cDNA was synthesized as previously described. qRT-PCR was performed using a CFX Connect real-time PCR system (Bio-Rad, Hercules, CA, USA) with 2× PolarSignal qPCR Mix (MIKX, MKG800-10, Shenzhen, China) following the instructions of the manufacturer. Each reaction mixture included 10 µL of 2× PolarSignal qPCR Mix, 1 µL of each primer (10 µM), and 1 µL of cDNA template in a total volume of 20 µL. The experiment was repeated three times, and three reactions were performed for each biological replicate. Gene expression levels were normalized to the β-actin expression level of the BPH [25]. Amplification was conducted as follows: 94 °C for 20 s, followed by 40 cycles of 94 °C for 10 s and 60 °C for 20 s. The melting curve was analyzed after amplification to verify primer specificity.

2.4. Measuring Body Weight of and Honeydew Produced by BPHs

One-day-old female BPHs were microinjected with either ds*NIGr23* or dsGFP and used for measurement. Their honeydew secretion was assessed through a protocol based on the method described in a previous study [12]. Parafilm membranes 5 cm long and 3 cm wide were preweighed using a sensitive balance. Subsequently, a single individual BPH was introduced into each sachet using fine forceps. The sachets were then affixed to the stems of the rice lines positioned 2 cm above the soil surface. Isolated BPHs were allowed to feed for 72 h. The weights of the bags in which both insects remained alive were measured again at the end of the feeding period. These live BPHs were individually weighed using a microbalance. The honeydew excreted from BPH nymphs was evaluated based on the difference in bag weight between before and after BPH feeding. Forty bag samples for each treatment were used to ensure that sufficient amounts of valid data were obtained for analyzing the differences (30 replicates). The entire experiment was repeated three times.

2.5. RNA-Seq Analysis

Total RNA was extracted from whole insects 48 h after dsRNA injection (Section 2.2). Three biological replicates per treatment (5 insects each) were used for RNA-seq. The mRNA was enriched with a poly (A) tail using magnetic beads containing oligo (dT), and the mRNA was interrupted with a buffer. To synthesize first-strand cDNA, we used fragmented mRNA as a template and random hexamer primers with an M-MuLV reverse-transcriptase system. Subsequently, the RNA strand was degraded using RNase H, and the second-strand cDNA was synthesized using dNTPs as the raw material in the DNA polymerase I system. Purified double-stranded cDNA was subjected to end repair, A-tailed addition, and sequencing. Library fragments were purified using an AMPure XP system (Beckman Coulter, Beverly, MA, USA) to select cDNA fragments 370–420 bp long. PCR was performed using Phusion High-Fidelity DNA polymerase (NEB, Beijing, China), Universal PCR primers (Beijing Balb, Beijing, China), and Index (X) Primer (Beijing Balb, Beijing,

China). The cDNA libraries were sequenced on an Illumina NovaSeq 6000 platform, and 150 bp paired-end reads were generated.

Raw data were preprocessed using SOAPnuke (v1.6.5) with parameters “-n 0.01 -l 15 -q 0.4 -G”, and clean reads were aligned to the reference genome (NCBI accession No. GCA_014356525.1) with HISAT2 (v2.2.1). Gene expression levels were quantified using RSEM (v1.3.1). DESeq2 (v1.22.1) was used to analyze differences in gene expression between groups. Genes with $|\log_2\text{foldchange}| \geq 1$ and a false discovery rate (FDR) < 0.05 were identified as significantly differentially expressed genes (DEGs). Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were performed for DEGs using Phyper (Q-value ≤ 0.05).

2.6. Protein Extraction and Trypsin Digestion

For proteomic analysis, whole insects were harvested 48 h post dsRNA injection (Section 2.2) and processed for protein extraction. Each treatment included three biological replicates with 10 insects pooled per replicate. The total protein was extracted from each group using a plant protein isolation kit (Qiagen, Hilden, Germany), following the protocol of the manufacturer. The purity and concentration of these isolated proteins were assessed using the Bradford protein assay [26]. 0.1 mg of protein sample was digested using mass spectrometry-grade trypsin (1:50 *w/w*, trypsin/protein) for 4 h at 37 °C. Peptides were desalted after digestion using a Strata X column to remove urea, and dried via vacuum centrifugation.

2.7. Proteomic Analysis

DIA data were corrected for retention time using indexed retention time (iRT) peptides. False positives were then controlled with an FDR ≤ 0.01 based on the target–decoy model applied using SWATH-MS to obtain significant quantitative results. The bioinformatics of the proteomic data were analyzed with MSstats (version 3.14.1) [27]. The thresholds of a $\log_2$ fold change (FC) ≥ 1 and an adjusted *p*-value < 0.05 were used to identify differentially expressed proteins (DEPs). All identified proteins were functionally annotated using GO (<http://geneontology.org/>) (accessed on 15 March 2024) and KEGG pathway analyses (<http://www.genome.jp/kegg/>) (accessed on 15 March 2024). DEPs were further subjected to GO and KEGG enrichment analyses.

2.8. Triacylglycerol, Glycerol, and Fatty Acid Assays

Samples were collected to determine the triacylglycerol (TAG) and glycerol contents in BPHs as follows. RNAi was quantified on one-day-old female BPHs that were used in follow-up experiments. Samples were collected 72 h after injection. Collected samples were stored at -80 °C after liquid nitrogen quick-freezing until further analysis. The TAG and glycerol contents were quantified as previously described [28]. The free fatty acid content was determined using copper soap colorimetry, according to the instructions of the manufacturer (Sangon Biotech, Shanghai, China). Each sample contained tissue extracts from five adult female BPHs, with three biological replicates per sample.

2.9. Statistical Analysis

Data were analyzed and visualized using GraphPad Prism software (version 8.0). Normality was confirmed with the Shapiro–Wilk test ($p > 0.05$). Two-group comparisons employed the Student’s *t*-test.

3. Results

3.1. Effect of *NIGr23* Knockdown on BPH Weight and Honeydew Production

NIGr23 mediates the repulsive responses of BPHs to oxalic acid in rice plants and artificial diets [25]. qRT-PCR analysis showed *NIGr23* transcript knockdown at 24 h, 48 h, and 72 h post-injection. No significant difference in efficiency was found between time points (Figure S1). Honeydew production and weight gain rates were measured following the injection of *dsNIGr23* to investigate the effects of *NIGr23* on BPH feeding. The body weight of and honeydew produced by BPHs substantially increased after the injection (Figure 1).

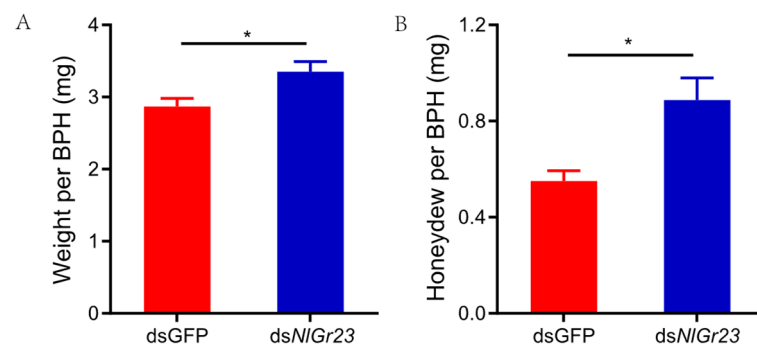


Figure 1. Effect of *NIGr23* knockdown on (A) weight of and (B) honeydew produced by BPHs. Data are presented as means $\pm$ SEM (n = 30). * indicates $p < 0.05$ (t-test).

3.2. Transcriptome Analysis of BPHs After Injecting *dsNIGr23*

We analyzed the RNA-seq of three biological replicate samples 48 h after injecting *dsNIGr23* to identify the underlying mechanisms. The total raw and clean data for each sample ranged from 42,091,806 to 46,022,766 bp and 41,481,222 to 45,202,956 bp, respectively (Table S2). More than 92.91% of the sequenced bases had a quality score of Q30 or higher. The total mapping rate of all samples to the reference genome ranged between 74.66% and 83.12% (Table S2), indicating that these data were usable for our subsequent analyses. A total of 11,247 genes were expressed in both the *dsNIGr23* and *dsGFP* groups, and 530 and 454 genes were identified in the *dsNIGr23* and *dsGFP* groups, respectively (Figure 2A). The Pearson correlation heatmap demonstrated low intergroup correlations, but high intragroup correlations, indicating the effects of *NIGr23* knockdown on gene expression patterns (Figure 2B). Clustering heat maps visually confirmed differences in the expression profiles between the groups (Figure 2C). These two groups contained 187 DEGs that met the $|\log_2\text{FC}| \geq 0.5$ and $p < 0.05$ thresholds, of which 156 genes were upregulated and 31 were downregulated (Figure 2C). The results of KEGG metabolic pathway enrichment analysis showed that these DEGs were primarily involved in the glycerophospholipid, cysteine, and methionine metabolism pathways (Figure 2D). These results suggested that *NIGr23* knockdown substantially affected multiple biological processes at the transcriptional level in BPHs.

3.3. Proteomics Analysis of BPHs After Injecting *dsNIGr23*

Proteomics reflect the effects of post-transcriptional regulation. Therefore, we used next-generation label-free quantitative proteomics technology to obtain the protein profile and identified 4496 proteins (Table S3). The length of most peptides in the proteomic data varied between 8 and 20 bp, indicating that the sample preparation met the standard (Figure 3A). InterProScan was used to analyze the gene family and structure of all detected proteins using Pfam, PRINTS, ProDom, SMART, ProSite, and PANTHER. A total of 1610 proteins were annotated using GO, the KEGG, Clusters of Orthologous Groups

(COG), and Interpro (IPR) (Figure 3B). Distinct protein expression clusters separated the dsGFP and ds*NIGr23* groups along the principal component analysis (PCA) axis (principal component 1 (PC1) = 48.28% and PC2 = 21.95%; Figure 3C). A total of 150 significant DEPs were identified, of which 79 were upregulated and 71 were downregulated (Figure 3D). The results of GO and KEGG analyses revealed that disrupting *NIGr23* in BPHs substantially altered their protein expression profiles (Figure 4). The GO analysis showed that biological processes were predominantly enriched in cholesterol metabolism and mono-organic substance transport. The enrichment was highest for the extracellular region and intercellular space, whereas molecular functions were strongly associated with copper ion-dependent enzymatic and peptidase activities (Figure 4A). The KEGG pathway analysis further demonstrated the significant enrichment of DEPs in critical metabolic and signaling networks, including glycerophospholipid, tyrosine, and fatty acid metabolism, as well as the AMPK signaling pathway (Figure 4B). These findings suggest that *NIGr23* disruption impairs cellular membrane function through disturbing lipid metabolic homeostasis, reducing energy supply efficiency through altered energy metabolism networks, and regulating cell survival via the activation of stress-responsive pathways such as the AMPK pathway.

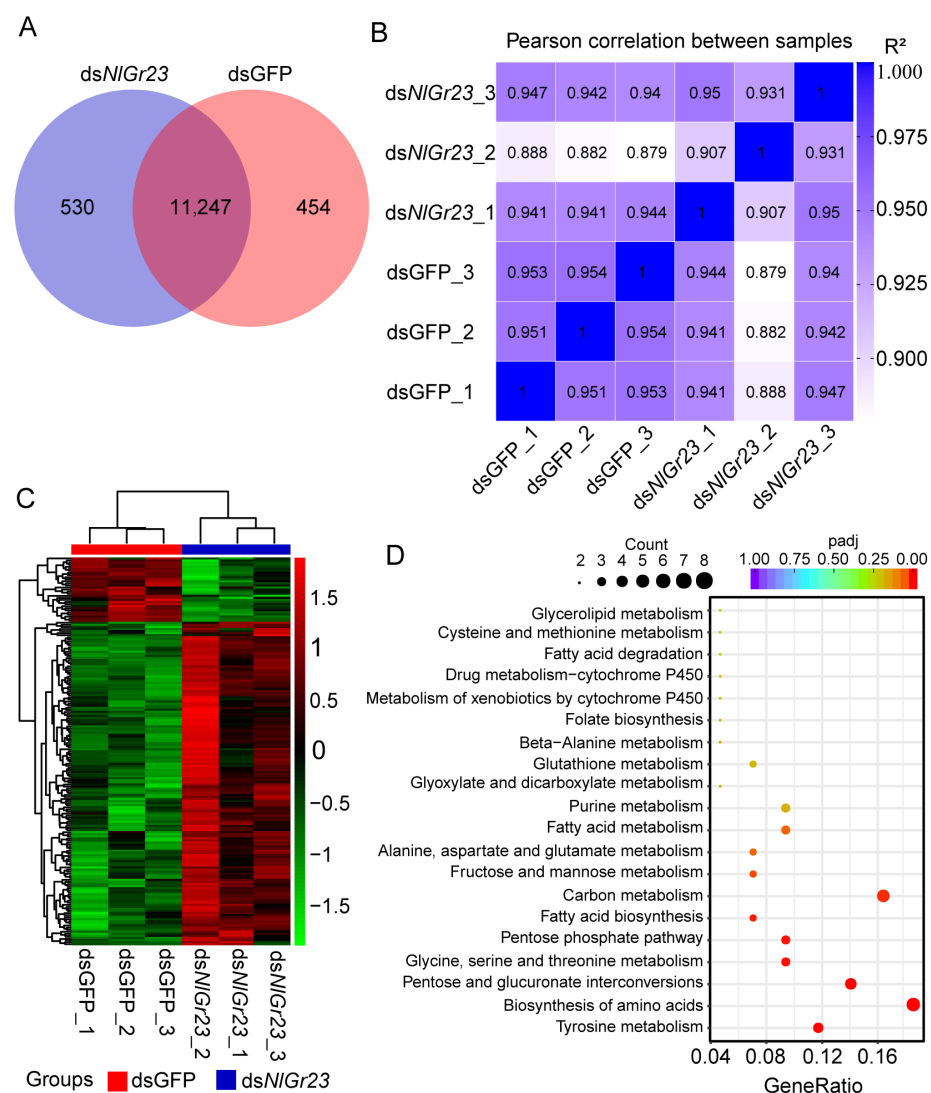


Figure 2. Transcriptome analysis of BPHs after injection with ds*NIGr23*. (A) Venn map of differentially expressed genes. (B) Results of correlation analysis between samples. (C) Heat map of differential gene clustering. (D) Kyoto Encyclopedia of Genes and Genomes enrichment analysis of differentially expressed genes.

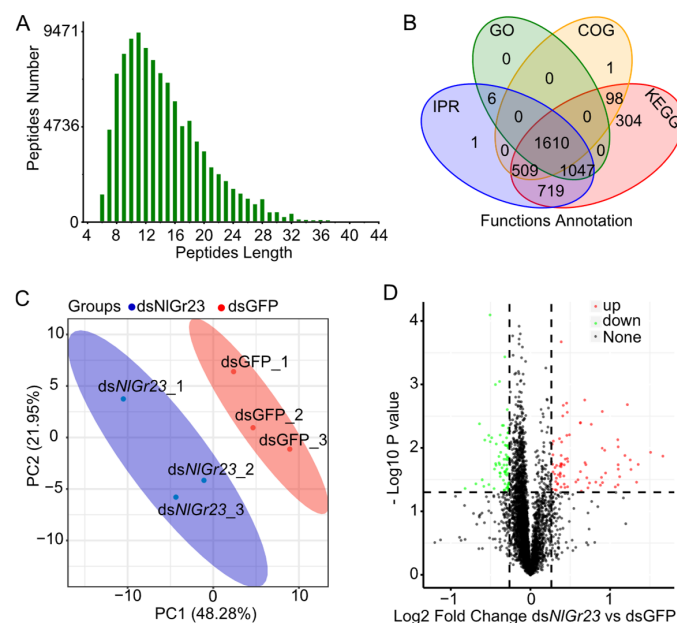


Figure 3. Proteomics analysis of BPHs after injection with dsNIGr23. (A) Relationship between peptide length and number; (B) Venn diagram of proteins annotated in different databases; (C) principal component analysis of proteomics; and (D) volcano plots showing the relative abundances of proteins (dsNIGr23 vs. dsGFP), p value < 0.05 , fold change < 0.83 or fold change > 1.2 , and $FDR \leq 0.05$ and $|\log_2(FC)| \geq 1$. Orange and green indicate proteins expressed are at higher and lower levels, respectively.

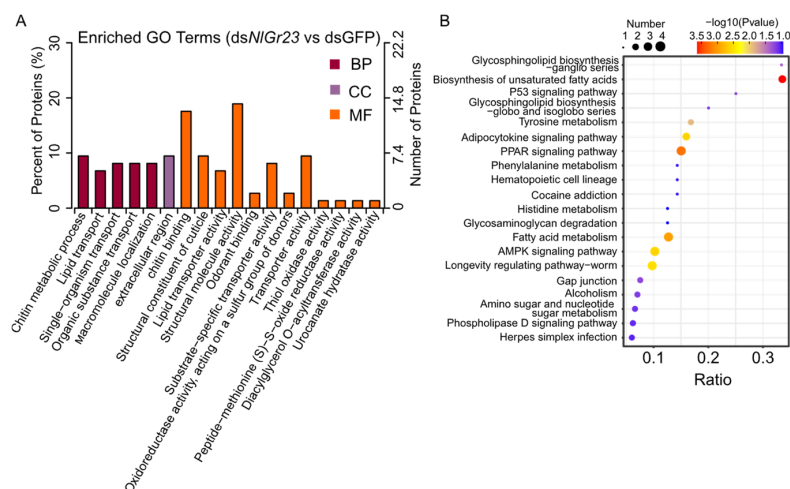


Figure 4. (A) Gene Ontology (GO) and (B) Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of differentially expressed proteins (DEPs).

3.4. Proteomics and Transcriptome Coexpression Analyses

The correlation of the relationship between proteomic and transcriptomic alterations during protein synthesis was analyzed through quantifying DEPs and DEGs (Figure 5). The Pearson correlation coefficient of transcriptomic and proteomic data was -0.293 , suggesting that some genes were significantly expressed at the mRNA level, but not at the protein level (Figure 5B). We identified 12 corresponding DEGs and DEPs (co-DEGs-DEPs) in the dsNIGr23 group compared with the dsGFP group. Among the 12 coexpressed DEGs and DEPs, the enriched pathways included metabolic pathways, amino acid biosynthesis, fatty acid metabolism, tyrosine metabolism, fatty acid biosynthesis, and glycine, serine, and threonine metabolism. This finding indicated that NIGr23 interference disrupted nutrient metabolism and synthesis, particularly fatty acid biosynthesis and metabolism, in BPHs.

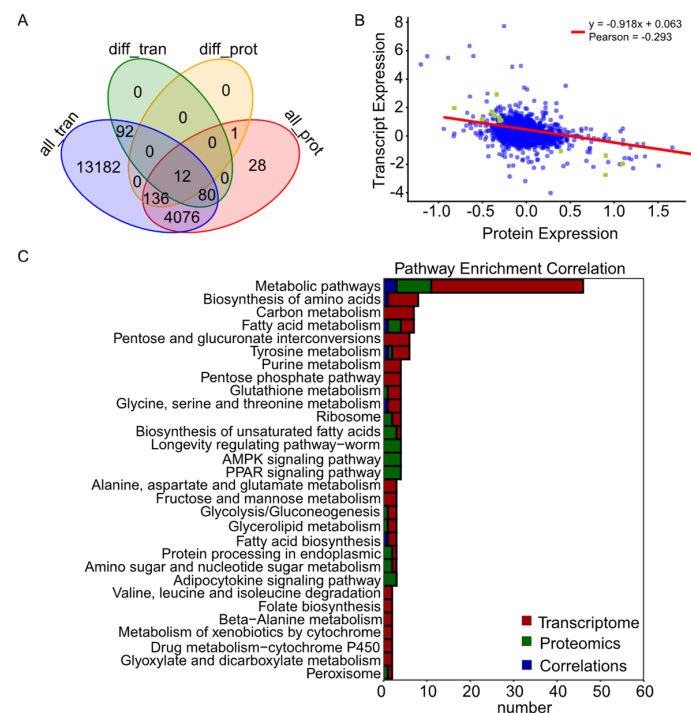


Figure 5. Joint proteomic and transcriptome analyses. **(A)** Combined analysis of Venn diagram; **(B)** Pearson's correlation between mRNA and protein expression levels. The dots in the graph indicate DEGs (blue) and DEPs (green); and **(C)** number of KEGG pathways enriched in correlation between transcriptome and proteome.

3.5. Effect of *NIGr23* Silencing on Lipid Mobilization

We hypothesized that increases in body weight and honeydew production in BPHs after *NIGr23* silencing resulted from disordered lipid metabolism and subsequent fat accumulation based on transcriptomic and proteomic results. We measured triglyceride levels in the *NIGr23*-silenced BPHs to test this hypothesis. The results showed *NIGr23* silencing substantially increased TAG levels by 68.83% (Figure 6A). In contrast, glycerol and free fatty acid contents were considerably lower in BPHs after *NIGr23* silencing compared with those of the wild type (Figure 6B,C).

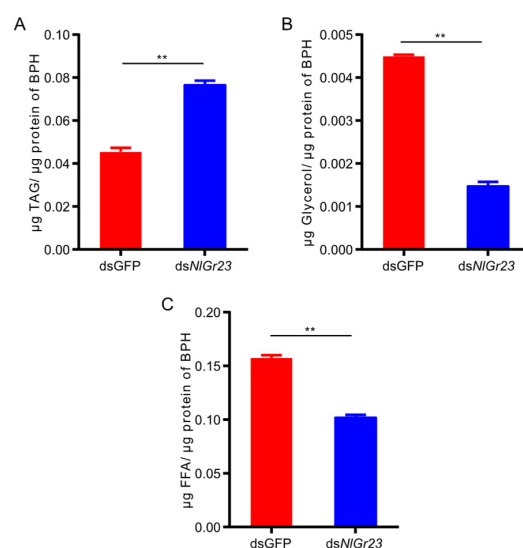


Figure 6. Effect of *NIGr23* silencing on lipid mobilization. **(A)** Triacylglycerol, **(B)** glycerol, and **(C)** free fatty acid contents in BPHs. ** Significant differences at 0.01 level (*t*-test). Data are presented as means $\pm$ SEM of at least three replicates.

4. Discussion

Insect GRs play pivotal roles in mediating feeding decisions and metabolic homeostasis. We found that silencing *NIGr23* in BPHs considerably increased feeding behavior by increasing honeydew excretion and body weight while disrupting lipid metabolism (Figure 1). The results of integrated transcriptomic and proteomic analyses revealed the molecular pathways underlying these phenotypic changes, highlighting the critical role of GRs in bridging chemosensation and metabolic regulation.

NIGr23 is a receptor that mediates repulsion with oxalic acid, a key deterrent in rice plants [25]. Our results showed that honeydew production and weight gain increased after *NIGr23* knockdown, indicating reduced aversion to host plant defenses. This aligns with the established roles of bitter-tuned GRs, such as *Drosophila* Gr66a, in recognizing toxins such as strychnine [29] and *Pieris rapae* Gr28 in detecting sinigrin [14]. The loss of aversive signaling likely explains the increased feeding, which is analogous to glucose-averse cockroaches, in which shifts in GR expression alter feeding preferences [30].

The multiomics predictions agreed with the findings of disrupted lipid homeostasis, which was experimentally confirmed using biochemical assays. *NIGr23* silencing increased triglyceride levels by 68.83% while depleting glycerol and free fatty acids (Figure 6), a phenotype signifying impaired lipolysis. We attributed this finding to AMPK activation, which suppressed lipases to promote lipid storage during energy deficit. Consequently, the limited free fatty acid availability restricted ATP synthesis, exacerbated metabolic inefficiency, and reduced insect fitness.

Our study identified a function of GRs in regulating systemic energy. GRs typically mediate neuronal activation [31]; *NIGr23* disruption directly reprograms the AMPK and lipid pathways. This disruption expands in parallel to that of sugar receptors, such as *NIGr7*, which suppress vitellogenin [16], or *Bombyx mori* Gr6/Gr9 in response to both sugars and plant stimulants [32]. We propose that *NIGr23* modulates neuroendocrine signaling or directly influences enterocyte metabolism. This study shows *NIGr23* silencing causes transcriptomic/proteomic changes in BPHs, but we cannot determine if these result directly from receptor loss or indirectly from increased oxalate intake.

Exploiting the GR-mediated metabolic pathways is an avenue for managing this pest. Compounds blocking *NIGr23* could enhance feeding in crops treated with low-toxicity deterrents such as oxalic acid, leveraging the behavioral and metabolic roles of the receptor. Alternatively, the activation of the AMPK or lipid storage pathways could induce energy deficiency without using conventional insecticides. These approaches align with emerging strategies for developing behavioral modulators that target GR networks.

5. Conclusions

We found that *NIGr23* silencing in the BPH disrupts lipid homeostasis via transcriptomic and proteomic reprogramming, culminating in the AMPK-mediated inhibition of lipolysis and TAG accumulation. We established GRs as key integrators of chemosensation and metabolism, providing a mechanistic basis for pest control strategies targeting GR-associated pathways. Future studies should explore the crosstalk between GRs and the neuroendocrine system and produce in vivo metabolic interventions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15081797/s1>, Figure S1: Interference efficiency of Gr23-RNAi in BPH. Shown are the means $\pm$ SD of three replicates, with five adults per replicate. * indicate significant differences in expression levels between dsGFP and ds*NIGr23*, as assessed using *t*-tests ($p < 0.05$).; Table S1: The primers used in this study; Table S2: Statistic of RNA-seq data; Table S3: Numbers of identified proteins in each sample.

Author Contributions: Conceptualization, K.K. and J.L.; methodology, K.K.; software, J.Z.; validation, K.K. and J.L.; formal analysis, R.F.; investigation, J.Z. and R.F.; resources, J.L.; data curation, K.K.; writing—original draft preparation, K.K.; writing—review and editing, J.L.; visualization, K.K.; supervision, J.L.; project administration, J.L.; funding acquisition, K.K. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets and analyses used during the current study are available in the NCBI Bioproject repository (PRJNA1279390).

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this paper:

BPH	Brown planthopper
GRs	Gustatory receptors
RNAi	RNA interference
cDNA	Complementary DNA
FDR	False discovery rate
DEGS	Differentially expressed genes
DEPs	Differentially expressed proteins
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
FC	Fold change
qRT-PCR	Quantitative reverse-transcription PCR
TAG	Triacylglycerol
iRT	Indexed retention time
COG	Clusters of Orthologous Groups
IPR	Interpro

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