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Regulatory networks of mRNAs and miRNAs involved in the immune response of diamondback moth, *Plutella xylostella* to fungal infection

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Abstract

Background The entomopathogenic fungus, *Isaria fumosorosea*, shows promise as a biological control agent in managing the diamondback moth (DBM) *Plutella xylostella,* a highly destructive global pest of cruciferous vegetables. To date, the miRNA-mRNA regulatory networks underlying the immune response of DBM to *I. fumosorosea* infection are still poorly understood. Here, we characterize the expression profiles of miRNA and mRNA, and construct the miRNA-gene regulatory network in DBM infected with *I. fumosorosea*.

Results We identified 580 differentially expressed genes (DEGs) and 55 differentially expressed miRNAs (DEMs) in *I. fumosorosea*-infected DBM. Among these DEGs, we found 28 immunity-related genes, which mainly include pattern recognition receptors, signal modulators, and immune effectors. Integrated analysis discovered 87 negative correlation pairs between miRNA and mRNA, involving 40 DEMs and 62 DEGs in infected DBM. Additionally, 13 miRNAs and 10 corresponding mRNAs were identified as candidate miRNA-mRNA pairs for DBM immunity against fungal infection. Gene functional enrichment analysis indicated that these miRNAs could target genes associated with various pathways, such as the immune system, infectious diseases, digestive system, endocrine system, nervous system, and signal transduction. Finally, the regulatory relationships of six miRNA-mRNA pairs were validated using quantitative reverse transcription PCR.

Conclusions For the first time, we present integrated miRNA and mRNA data to elucidate the immune response of the DBM to fungal infection. Our findings enhance the understanding of the immune response of the DBM to entomopathogenic fungi infection.

Keywords *Plutella xylostella*, *Isaria fumosorosea*, miRNA-mRNA regulatory networks, Immune response

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Background

The diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae), poses a significant threat to cruciferous crops worldwide. It develops resistance to nearly all commonly used pesticides for its control [1, 2]. While chemical control remains the primary approach to control DBM [3], it is urgent to develop alternative, environmentally friendly, and sustainable control strategies. The entomopathogenic fungi, *Isaria fumosorosea*, has emerged as potential biocontrol agents for various insect species [4, 5]. The *I. fumosorosea* strain IFCF01 used in this study demonstrates high pathogenicity to DBM [6]. It's spores extensively infiltrated the body cavity of the DBM within 24 h after treatment by a concentration of 1×10^7 spores/mL *I. fumosorosea* [7]. However, the slower action exhibited when used in the field by the fungal pathogens in comparison to chemical pesticides hinders the widespread utilization of fungal insecticides [8]. Insects have evolved remarkable adaptability and possess a unique innate immune system that helps them defense against fungal infections [9]. To enhance the efficacy of fungal-based treatments, it is crucial to understand the host;s immune responses triggered by fungi infection. Upon infection by fungi, insects possess the ability to recognize pathogen-associated molecular patterns (PAMP) which then triggers both cellular and humoral immune responses [10]. These responses include blood cell-mediated phagocytosis, envelopment, and the production of antimicrobial peptides [11, 12].

Functioning as important regulators, miRNAs exert a negative influence on the expression of target genes at the transcriptional or post-transcriptional levels [13]. Research has demonstrated that the interaction between miRNA and mRNA is crucial in various biological processes in insects, including growth and development, immune response, and host–pathogen interactions [14–18]. Previous studies have predominantly focused on the individual impact of miRNAs and mRNAs during fungal infection in DBM [5, 19]. However, integrating transcriptional and post-transcriptional datasets to uncover the co-regulatory mechanisms is currently an important area of research. Some studies have explored the combined regulatory mechanisms of miRNA and mRNA in response to insecticide or gut microbiota in insects. It has been reported that mRNA and its target genes play a crucial role in mediating the interaction between the gut microbiota and its host *Bactrocera citri*. In *Lymantria dispar*, miRNAs and their target genes are closely linked to the tolerance to insecticide cyantraniliprole [16, 17]. To our knowledge, the combined regulatory mechanisms of miRNA and mRNA in response to fungal infection in DBM remain largely unknown.

In this study, we investigate the response networks regulated by miRNA and mRNA to gain a comprehensive understanding of the immune response of DBM against *I. fumosorosea* infection. This is the first attempt to explore the potential relationship between miRNA-regulated genes and insect immunity to fungal infections. It provides a valuable reference for understanding the miRNA-mediated regulatory mechanism of DBM immunity to fungi, and also serves as an important reference for biological control of insect pests through the regulation of insect immune system.

Materials and methods

Insect rearing

The DBM strain utilized in this study was obtained from Yichun, Jiangxi Province, in 2019 and was reared for more than 30 generations at the Jiangxi Key Laboratory of Crop Growth Regulation, Yichun University. The DBMs were maintained at a relative humidity of 70% and a constant temperature of 27 ± 1 °C, following a light:dark cycle of 14 h to 10 h. Adult insects were provided with a 5% honey solution, while the larvae were fed with Chinese kale (Brassica oleracea var. alboglabra).

Bioassay and sample collection

The *I. fumosorosea* strain IFCF01 (China Center for Type Culture Collection access number: CCTCC M 2013526), provided by the Biological Control Laboratory of South China Agricultural University, was preserved in tubes with 20% glycerin and stored at -80 °C in Yichun University. The fungal strain IFCF01 was cultured on potato dextrose agar for 7 days at a temperature of 25 ± 1 °C in complete darkness. Conidia were collected using sterile water containing 0.05% Tween-80 and filtered through filter paper. The concentration of the conidia suspension was adjusted to 1×10^7 conidia/mL using a hemocytometer with a 40× objective microscope (OLYMPUS BX43F, Japan). Healthy second- and third-instar larvae of DBM were treated with a suspension of 1×10^7 spores/mL and subsequently reared on fresh Chinese kale leaves. About 24 h after treatment when the infection reach to a a relatively significant level [7], 15 2nd to 3rd instar larvae were collected from each replicate and stored at -80 °C until RNA isolation. In the control group, larvae were treated with sterile water containing 0.05% Tween-80.

mRNA and miRNA library construction and sequencing

Total RNA were isolated using the Trizol reagent Kit (Invitrogen, Carlsbad, CA, USA). The quality and concentration of RNA were evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), Nanodrop2000 Spectrophotometer, and RNase-free agarose gel electrophoresis. The Illumina Ribo-Zero Gold

reagent kit (MRZG12324, Illumina) was used to construct mRNA libraries. The mRNAs were fragmented into shorter fragments employing a fragmentation buffer, and then reverse-transcribed into cDNA using random primers. Subsequently, second-strand cDNA synthesis took place using DNA polymerase I, RNase H, dNTP (with dUTP instead of dTTP), and buffer. The resulting cDNA fragments were purified through the QIAquick PCR purification kit (Qiagen, Venlo, The Netherlands). Next, the fragments underwent end repair, poly(A) addition, and ligation to Illumina sequencing adapters. Uracil-N-Glycosylase (UNG) was utilized to digest the second-strand cDNA. The products underwent size selection through agarose gel electrophoresis, PCR amplification, and sequencing on an Illumina HiSeq™ 4000 at Gene Denovo Biotechnology Co. (Guangzhou, China).

The miRNA libraries were generated using the MiRNA Sample PreKit (Illumina, San Diego, CA, USA). Initially, RNA molecules in the size range of 18–30 nt were enriched from total RNA through polyacrylamide gel electrophoresis. Subsequently, 3' adapters were introduced via T4 RNA ligase to enrich the 36–44 nt RNAs. Moreover, 5' adapters were ligated to the RNAs. The resulting ligation products were subjected to reverse transcription through PCR amplification, and the PCR products, with a size of 140 ~ 160 bp, were further enriched to generate a complementary DNA (cDNA) library. The libraries were subsequently sequenced on the Illumina HiSeqTM 2500 (Illumina, San Diego, CA, USA) by Gene Denovo Biotechnology Co. (Guangzhou, China).

Analysis of transcriptome data

The raw sequencing reads were filtered using fastp v0.18.0 [20]. This involved the removal of reads containing adapters, eliminating reads with over 10% unknown nucleotides (*N*), and discarding low-quality reads with more than 50% of bases with low quality (*Q*-value ≤ 20). Alignment of the clean reads with the reference genome of DBM (GCA_000330985.1) was performed using HISAT2 [21]. Transcript abundances were then determined using the reference-based approach provided by StringTie v1.3.4. FPKM (fragments per kilobase of transcript per million mapped reads) values were utilized to quantify the expression of the transcripts. Differential expression analysis was conducted using DESeq2 [22]. Genes meeting the criteria of a false discovery rate < 0.05 and an absolute fold change > = 2 were considered differentially expressed genes (DEGs). Subsequently, enrichment analysis for Gene Ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were performed for the identified DEGs.

Analysis of miRNA data

The raw sequence output data underwent processing using the Genome Analyzer Pipeline Software (Illumina). This involved the removal of low-quality sequences, 5' primer contaminants, 3' primers, insert tags, and sequences shorter than 18 nucleotides. The remaining high-quality, clean tags were then aligned to miRNAs in the GenBank (Release 209.0) and Rfam database (Release 11.0) to effectively identify and eliminate rRNA, scRNA, snoRNA, snRNA, and tRNA [23]. Subsequently, these sequences were mapped to the DBM genome (GenBank accession number: GCF_905116875.1) using Bowtie v1.2.2 to exclude tags that corresponded to exons, introns, and repeat sequences. The remaining tags were cross-referenced with the miRBase database (Release 21.0) to identify the presence of known miRNAs specific to DBM, namely existing miRNA. Furthermore, miRNAs with alignments to other species were also identified, namely known miRNA. The remaining unannotated clean sequences were subjected to miRDeep2 software [24] for the prediction of novel miRNAs. To calculate the expression level of each miRNA, normalization to transcripts per million (TPM) was performed using the formula: TPM = (number of mapped reads for each miRNA/total number of mapped reads) $\times 10^6$. The identification of DEMs was achieved using the edgeR package [25], with DEMs selected based on log2(fold change) > 1 and *P*-values < 0.05.

Target prediction of differentially expressed miRNAs

The candidate target genes were identified from the DEGs between *I. fumosorosea*-infected and non-infected DBMs. To predict the target genes, miRanda v3.3a, TargetScan v7.0, and RNAhybrid v2.1.2 were employed, utilizing their default parameters. The resulting list of candidate miRNA-targeted genes was obtained through the intersection of these three analyses.

Construction of miRNA-mRNA regulatory network

To investigate the regulatory relationship between miRNAs and their target genes, Pearson correlation analysis was conducted to analyze the correlation between miRNA and mRNA expressions. A correlation threshold of < -0.6 and $p < 0.05$ was applied to identify negatively co-expressed miRNA-target mRNA pairs. Furthermore, GO and KEGG enrichment analysis was performed to explore the biological processes associated with miRNA-regulated target genes involved in the response of DBM to fungal infection. Finally, the miRNA-mRNA regulatory network was constructed using Cytoscape v3.9.1.

RT-qPCR validation

To validate the gene expression profiles obtained from Illumina sequencing, we conducted RT-qPCR to determine the expression of six miRNAs and their corresponding target genes. Total RNA was extracted from the same larval samples as used for RNA sequencing. The All-in-One miRNA First-Strand cDNA Synthesis Kit 2.0 (FulenGen, Guangzhou, China) and the Hifair®II 1st Strand cDNA Synthesis Kit (Yeasen Biotechnology, Shanghai, China) were used to synthesize the first-strand cDNA for miRNA and mRNA, respectively. RT-qPCR was performed on a Bio-Rad T100 Real-Time PCR system (Bio-Rad) utilizing the Hieff® qPCR SYBR Green Master Mix (Low Rox Plus) as recommended by the manufacturer (Yeasen Biotechnology, Shanghai, China). Each RT-qPCR reaction was replicated three times with three technical replications under the following conditions: an initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 10 s and annealing/extension at 62 °C for 30 s, and a melting curve analysis (60 °C-95 °C). The specificity of amplification was confirmed by analyzing the melting curves for each sample after each run. The expression of miRNA and mRNA was normalized to U6 and eEF1beta, respectively. The primers for the target genes were designed using Primer Premier 5 (Table S1). Relative expression was determined using the $2^{-\Delta\Delta Ct}$ method, and the fold change was transformed to log2 values to represent the differential expression of mRNA or miRNA between the treatment group and the control group. Between-group comparisons were analyzed using one-way ANOVA followed by Tukey's multiple comparison tests. SPSS 16.0 was used for all statistical analyses, with a significant level of 0.05.

Results

DEGs in *I. fumosorosea*-infected DBM

Following quality control procedures, the mRNA libraries of *I. fumosorosea*-infected and non-infected DBMs produced about 272 million and 286 million clean reads, respectively, for each of the three replicates. The Q20 value exceeds 97.0%, and the Q30 value is above 92.0%. Approximately 85.98% to 89.33% of the reads were mapped to the DBM genome, with 79.51% to 82.83% being uniquely mapped (Table S2). The sequencing reads were deposited in the Sequence Read Archive (SRA) of NCBI (Bioproject accession number: PRJNA1050803).

A total of 580 DEGs were identified in the comparison between *I. fumosorosea* infected and non-infected DBMs, consisting of 384 up-regulated genes and 196 down-regulated genes (Fig. 1, Table S3A). The GO enrichment analysis identified 414 DEGs between infected and non-infected DBMs and classified these DEGs into three main categories, comprising 61 subcategories at level 2. These subcategories included 26 related to Biological Process, 12 related to Molecular Function, and 23 related to Cellular Component. The majority of the DEGs were mainly

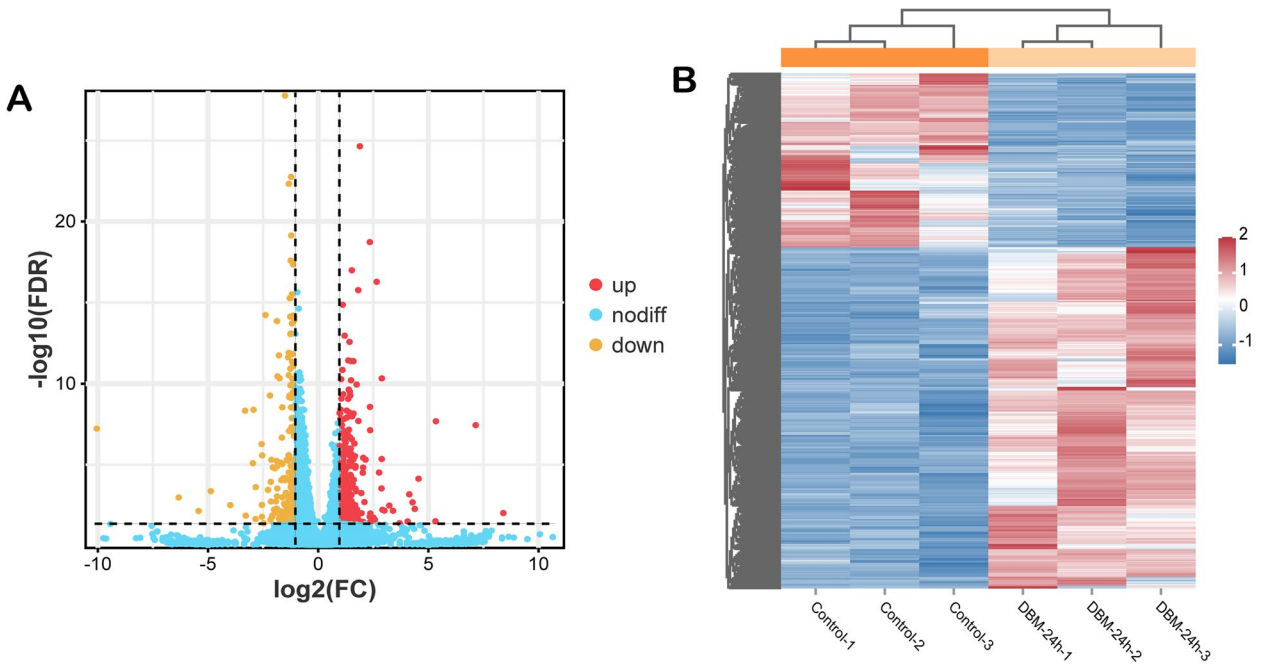
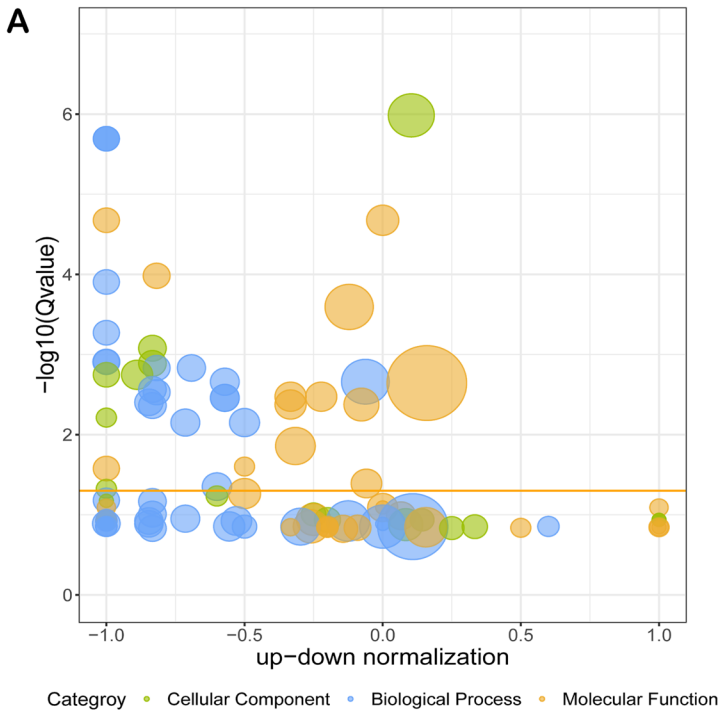


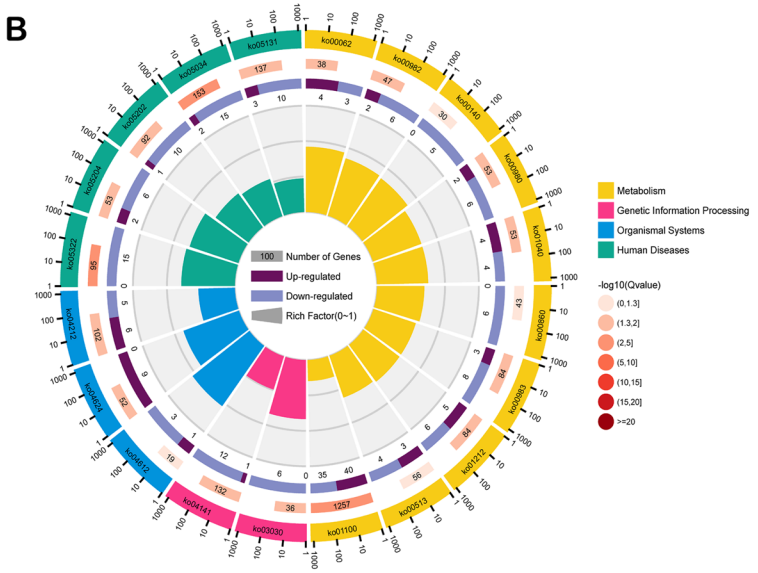
Fig. 1 Overview of differentially expressed genes (DEGs). **A** Volcano of DEGs. **B** Profile of DEGs in *Plutella xylostella* infected with *Isaria fumosorosea* compared with noninfected (control) *P. xylostella*

enriched in GO terms associated with "cellular process", "single-organism process", "metabolic process", "binding", and "catalytic activity" (Table S3B). The top 20 GO terms (level 3) related to *I. fumosorosea* infection are shown in Fig. 2A. The KEGG enrichment analysis revealed that 195 DEGs were annotated across 275 pathways at level 3



GO term Top 20

ID	Descrption
GO:0005576	extracellular region
GO:0038111	interleukin−7−mediated signaling pathway
GO:0098760	response to interleukin−7
GO:0098761	cellular response to interleukin−7
GO:0042302	structural constituent of cuticle
GO:0031492	nucleosomal DNA binding
GO:0031491	nucleosome binding
GO:0000183	chromatin silencing at rDNA
GO:0016491	oxidoreductase activity
GO:0045652	regulation of megakaryocyte differentiation
GO:0000786	nucleosome
GO:1901532	regulation of hematopoietic progenitor cell differentiation
GO:0030219	megakaryocyte differentiation
GO:0044815	DNA packaging complex
GO:0060964	regulation of gene silencing by miRNA
GO:0002244	hematopoietic progenitor cell differentiation
GO:0032993	protein−DNA complex
GO:0071821	FANCM−MHF complex
GO:0007596	blood coagulation
GO:0055114	oxidation−reduction process



Pathway ID	Pathway name
ko05322	Systemic lupus erythematosus
ko01100	Metabolic pathways
ko05034	Alcoholism
ko04624	Toll and Imd signaling pathway
ko00983	Drug metabolism - other enzymes
ko01212	Fatty acid metabolism
ko00982	Drug metabolism - cytochrome P450
ko00062	Fatty acid elongation
ko05202	Transcriptional misregulation in cancers
ko00980	Metabolism of xenobiotics by cytochrome P450
ko01040	Biosynthesis of unsaturated fatty acids
ko05204	Chemical carcinogenesis
ko04141	Protein processing in endoplasmic reticulum
ko04212	Longevity regulating pathway - worm
ko03030	DNA replication
ko05131	Shigellosis
ko04612	Antigen processing and presentation
ko00140	Steroid hormone biosynthesis
ko00513	Various types of N-glycan biosynthesis
ko00860	Porphyrin and chlorophyll metabolism

Fig. 2 Enrichment Analysis of GO Terms and KEGG Pathways for Differentially Expressed Genes (DEGs). **A** The GO bubble plot provides a summary of the top 20 enriched GO terms at level 3. Various colors denote distinct GO categories. The x-axis reflects the normalization of up- and down-regulation, while the y-axis indicates the And the y-axis represents negative log10 (*Q* value). **B** A circular plot illustrates the results of KEGG enrichment analysis. The outermost circle represents the top 20 KEGG pathways at level 3, with the number of genes corresponding to the outer perimeter. The subsequent circle displays the number of genes in the genome background and the *q*-values indicating the significance of DEG enrichment for specific biological processes. The color intensity signifies the enrichment significance: deeper red hues correspond to smaller *q*-values. The third circle depicts the proportion of up-regulated genes (shown in dark purple) and down-regulated genes (displayed in light purple). The innermost circle indicates the enrichment factor for each KEGG term

(Table S3C). The top 20 KEGG pathways related to fungus infection were categorized into four major groups: Metabolism, Genitic Information Processing, Organismal Systerm, and Human Disease (Fig. 2B). The three most enriched pathways were systemic lupus erythematosus, metabolic pathways, and alcoholism. Notably, the Toll and IMD signaling pathways related to innate immune response against microbial infection include 9 up-regulated expression genes (Fig. 2B).

Identification of differentially expressed immunity-related genes

In order to identify immune-related genes against *I. fumosorosea* infection in DBM, we conducted a comprehensive analysis using a combination of BLAST search, GO and KEGG annotation results, and published studies. We excluded differentially expressed genes that were annotated as hypothetical or unknown proteins, resulting in the identification of a total of 28 immune-related genes (Table 1). These immune-related genes were classified into immune recognition families, signal modulation, immune signaling pathways, immune effectors and others.

DEMs and their target genes

Following quality control, the miRNA libraries of three replicates for both *I. fumosorosea* noninfected and infected DBMs resulted in the generation of the following clean reads: 8,199,838, 8,235,566, and 7,795,249 for infected DBMs and 8,481,954, 9,372,420, and 7,059,133 for noninfected DBMs, respectively (Table S4). Approximately 32.46% to 67.75% of these reads were successfully mapped to the DBM genome. The corresponding sequencing reads have been deposited in the SRA of the NCBI (Bioproject accession number PRJNA1051778).

We identified 55 DEMs between noninfected and infected DBMs. Among these, four were existing miRNAs of DBM, 43 were known miRNAs of other species, and 12 were novel miRNAs. Out of the 55 DEMs, 40 miRNAs (including 34 known, 1 existing, and 5 novel) exhibited up-regulation, while 15 miRNAs (including 9 known, 3 existing, and 3 novel) displayed down-regulation (Fig. 3; Table S5). The analysis using RNAhybrid v2.1.2, miRanda v3.3a, and TargetScan v7.0 revealed 254 miRNA-mRNA interactions, involving 53 differentially expressed miRNAs and 141 target mRNAs (Fig. S1; Table S6).

miRNA and mRNA interaction network construction

A total of 87 negatively correlated miRNA-mRNA pairs were identified, involving 40 DEMs and 62 DEGs (Table S7). Among these pairs, 81 were known interactions between miRNAs and mRNAs, and six were novel interactions. MicroRNA primarily exerts a negative regulatory function by binding to the 3' UTR region of target genes, promoting mRNA degradation or inhibiting protein translation. Based on this characteristic, the correlation between DEM and DEG was analyzed, resulting in the identification of two miRNA-mRNA networks. The first network included 26 up-regulated DEMs and 31 down-regulated DEGs (Fig. 4A), while the second network consisted of 12 down-regulated DEMs and 30 up-regulated DEGs (Fig. 4B). It is worth noting that a single miRNA can target multiple genes. For example, the up-regulated miR-5119-z was found to target six mRNAs, including C05D11.1, dpa, Sox21, Gld, ncbi_105382220, and ncbi_105396991. Conversely, the down-regulated miR-305-x was predicted to target TBC1D19 (TBC1 domain family member 19 isoform X1), norpA (1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like), CG3781 (josephin-2), slmo (protein slowmo), chico (uncharacterized protein LOC105385992), and other genes. Moreover, multiple miRNAs could regulate one mRNA. For instance, the gene ds (protein dachsous) was targeted by miR-1494-z, miR-12036-z, and miR-6497-x; the TKFC (triokinase/FMN cyclase-like) gene was targeted by miR-10018-x and miR-3759-y; the Prdm1 (PR domain zinc finger protein 1 isoform X1) gene was a target of miR-8503-z, miR-29-x, and miR-214-y; and the NitFhit (nitrilase homolog 1-like) gene was targeted by miR-214-y, miR-6498-x, and miR-10298-z. A comprehensive list of all miRNA-mRNA pairs potentially involved in the immune response against fungal infection is detailed in Table S7.

Functional characterization of miRNA negatively regulated target genes

To gain further insight into the potential biological functions of target DEGs of miRNAs, GO and KEGG analyses were performed. The GO function analysis revealed the enrichment of three GO categories, which consisted of 50 subcategories at level 2. The categories of cellular components showed that cell, and cell part were the most enriched terms, while the categories of molecular function indicated that catalytic activity and binding were the top enriched terms. In the categories of biological process, the main terms were single-organism process and cellular process (Fig. 5). Specifically, 21 target genes were annotated with the GO term response to stimulus, 5 with immune system process, 2 with antioxidant activity, and 27 with catalytic activity. These annotated genes potentially play significant roles in the immune responses of DBM to fungal infections (Fig. 5; Table S8A).

The top 20 KEGG pathways of miRNA negatively regulated genes were classified into five major groups: environmental information processing, genetic information processing, human diseases, metabolism, and

Table 1 Summary of differentially expressed immunity-related genes

Gene ID	Name	log2(FC)	References
Immune recognition families			
Peptidoglycan recognition protein			
ncbi_105391041	peptidoglycan recognition protein PGRP-S2	1.07	Xu et al., [5]
ncbi_105387860	peptidoglycan-recognition protein LB-like	1.34	Xu et al., [5]
Signal Modulation			
RAC serine/threonine-protein kinase			
ncbi_105380907	RAC serine/threonine-protein kinase	1.01	Ai et al., [26]
Serine protease snake-like			
ncbi_105380881	serine protease snake-like	1.03	Wang et al., [27]
Serine protease easter-like			
ncbi_119690994	serine protease easter-like	1.03	Wang et al., [27]
Serine/threonine-protein kinase RIO1			
ncbi_105398499	serine/threonine-protein kinase RIO1	1.01	Li et al., [28]
Venom serine carboxypeptidase-like			
Metalloproteinase inhibitor			
ncbi_105388015	metalloproteinase inhibitor 3	1.05	Vertyporokh et al., [29]
Immune signaling pathways			
modular serine protease			
ncbi_105390003	modular serine protease	1.13	Buchon et al., [30]
Serine/threonine-protein kinase pelle-like			
ncbi_105390192	serine/threonine-protein kinase pelle-like	1.07	Wu et al., [31]
Immune effectors			
Apolipophorins			
ncbi_105380513	apolipophorins-like	-1.28	Wang et al., [27]
ncbi_119693854	apolipophorin-III	-1.09	Wang et al., [27]
Gloverin			
ncbi_105389803	gloverin	1.28	Wang et al., [27]
Cecropin			
ncbi_105394860	cecropin1	2.69	Xu et al., [5]
ncbi_105394859	cecropin2	2.92	Xu et al., [5]
ncbi_105392561	cecropin3	2.91	Xu et al., [5]
ncbi_105394858	cecropin4	3.26	Xu et al., [5]
Prophenoloxidase activating proteinase			
ncbi_105393827	prophenoloxidase activating proteinase 3	1.17	Kanost et al.,[32]
ncbi_105384195	prophenoloxidase activating proteinase 1	1.07	Kanost et al.,[32]
Others			
Superoxide dismutase			
ncbi_105383124	superoxide dismutase	1.17	Xia et al., [33]
Peroxidase-like			
ncbi_105388497	peroxidase-like	-1.02	Xia et al., [33]
Transferrin			
ncbi_105384728	pxTransferrin	1.54	Xu et al., [5]
Thioredoxin domain-containing protein 12-like			
ncbi_105382641	thioredoxin domain-containing protein 12-like	1.50	Thulasitha et al., [34]
A disintegrin and metalloproteinase with thrombospondin motifs 16-like			
ncbi_105380255	A disintegrin and metalloproteinase with thrombospondin motifs 16-like	-1.15	Kelwick et al., [35]
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like			
ncbi_105388539	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like	1.04	Huang et al., [36]

Table 1 (continued)

Gene ID	Name	log2(FC)	References
Lysosomal thioesterase PPT2 homolog			
ncbi_105398668	lysosomal thioesterase PPT2 homolog	1.05	Xu et al., [5]
Plasminogen activator inhibitor 1-like			
ncbi_105390553	plasminogen activator inhibitor 1-like	1.08	Yadav et al., [37]
Protein disulfide-isomerase			
ncbi_105393053	protein disulfide-isomerase A6	-1.19	Liu et al., [38]
ncbi_105391385	protein disulfide-isomerase A3	-1.29	Liu et al., [38]

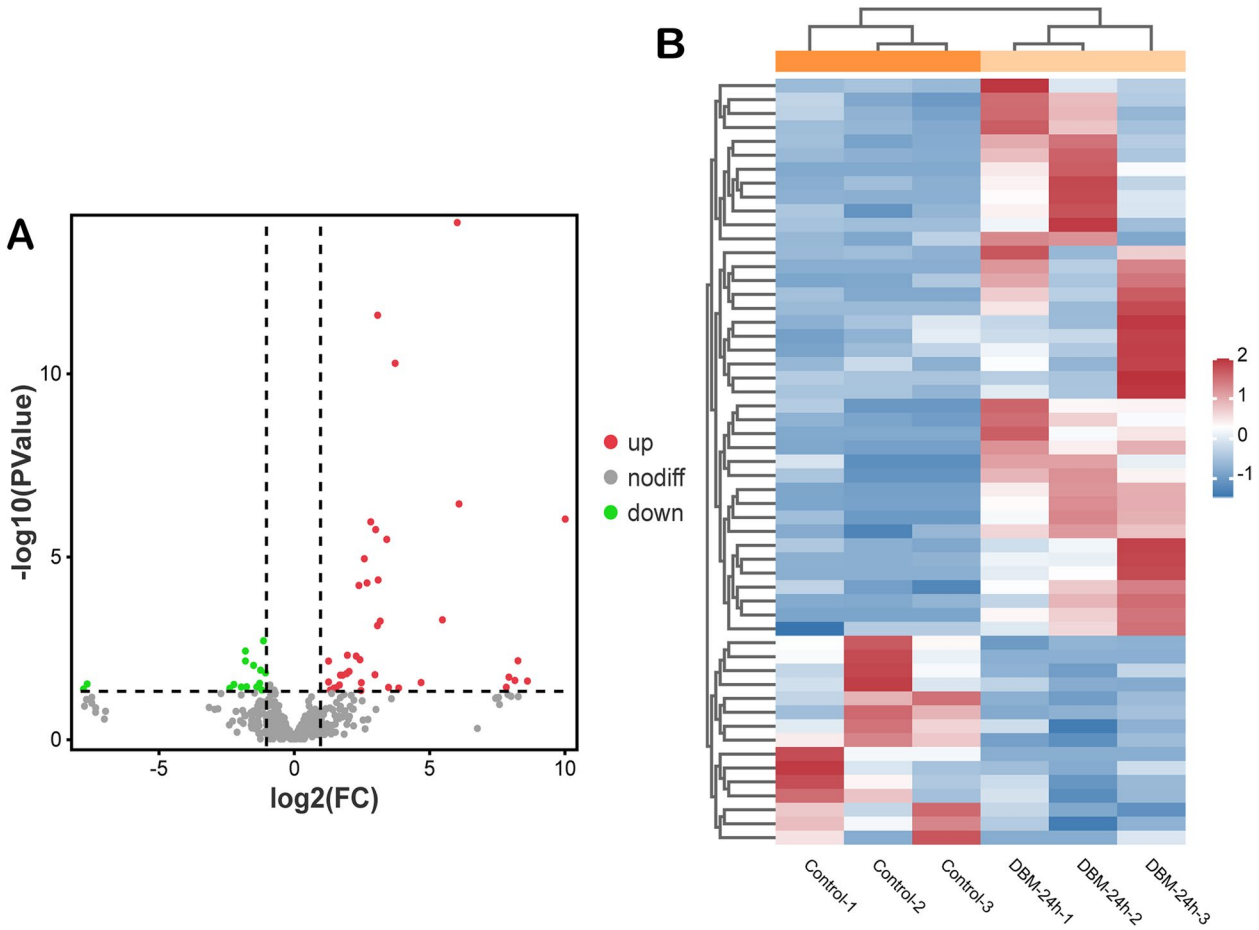


Fig. 3 Differential Expression Profiles of microRNAs (DEMs). **A** Volcano Plot of DEMs. **B** The expression profiles of DEMs in *Isaria fumosorosea* infected (DBM-24 h) and noninfected (control) *Plutella xylostella*

organismal systems (Level 1) (Fig. 6A). Further analysis revealed enrichment mainly in categories such as the immune system, digestive system, cancer, endocrine system, infectious diseases, nervous system, signal transduction, and endocrine and metabolic diseases (Level 2) (Fig. 6B). Specifically, miR-1298-x targets the Akt1 gene, which is associated with eight immune-related pathways. Both miR-305-z and miR-305-x were identified as regulators of the norpA and chico genes, with both genes concurrently linked to multiple immune-related pathways. Furthermore, miR-750-y targets the PDIA3 gene, which is associated with both immune system and infectious disease pathways. The target genes of miR-10298-z and novel-m0160-3p

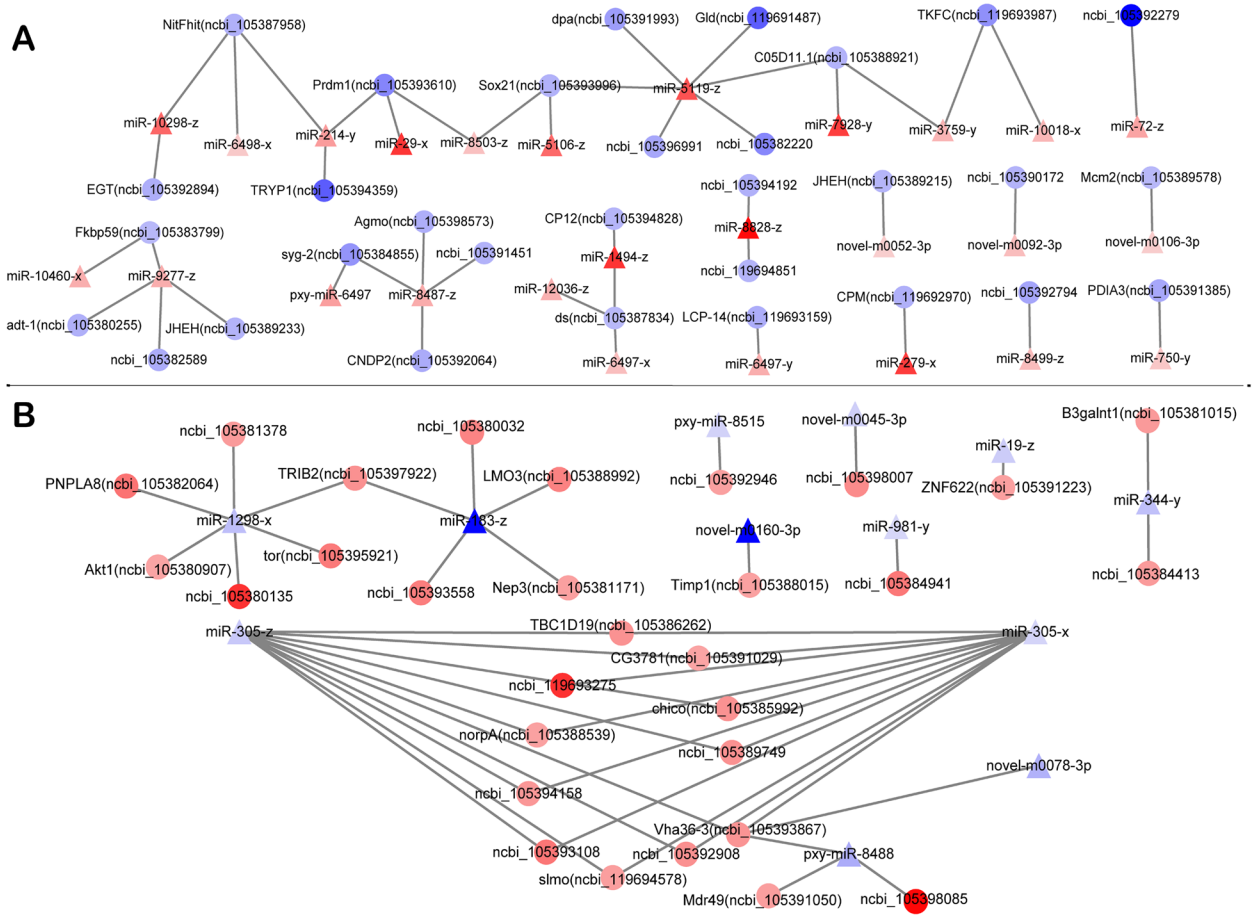


Fig. 4 Interaction Network Analysis of miRNAs and mRNAs. **A** The miRNA-mRNA network consists of 26 up-regulated DEMs and 31 down-regulated DEGs. **B** Another network of miRNA-mRNA was constructed with 12 down-regulated DEMs and 30 up-regulated DEGs. Up-regulated genes (both miRNAs and mRNAs) are colored red, while down-regulated genes (both miRNAs and mRNAs) are colored blue. The symbols used are as follows: △ represents miRNAs, and ○ represents mRNAs

were found to be enriched in cancer pathways. Additionally, pxy-miR-8488 targets the Mdr49 gene, which is associated with cancer and digestive pathways. miR-214-y targets the TRYP1 gene, which is annotated to the digestive system and infectious disease pathways. Meanwhile, miR-12036-z, miR-1494-z, and miR-6497-x all target ds genes annotated to the signal transduction pathway. Furthermore, miR-10460-x and miR-9277-z were found to simultaneously target the Fkbp59 gene, which is enriched in the endocrine system pathway. The above 13 identified miRNAs and their corresponding 10 mRNAs represent potential miRNA-mRNA relationship pairs that may be crucial for the immunity of DBM to fungal infections. It is worth noting that our study found that four negatively correlated miRNA-mRNA pairs, which include 4 miRNAs and 3 mRNAs, that are associated with the immune system. In all, the DEGs negatively regulated by miRNAs were enriched in 163 pathways at level 3 (Table S8B).

Validation of DEMs and their potential targets by RT-qPCR

The expression analysis of six miRNA-mRNA pairs was conducted, revealing the following results: the expression of miR-10018-x, as determined by RT-qPCR, showed a 1.01-fold increase, while its target gene TKFC exhibited a 1.35-fold decrease. A negative correlation was observed between the down-regulated expression of miR-1298-x and its target gene Akt1. Both miR-305-x and miR-305-z showed down-regulated expression, whereas their target gene norpA displayed up-regulation. Meanwhile, miR-750-y demonstrated a 1.11-fold increase in expression, whereas its target gene PDIA3 showed a 1.09-fold down-regulation. Similarly, a negative correlation was observed between the up-regulated expression of miR-6497-y and its target gene LCP-14. Importantly, these results were consistent with the trend observed in the miRNA-seq and mRNA-seq data (Fig. 7). The findings of our study provide evidence for a negative correlation between miRNA and target gene expressions.

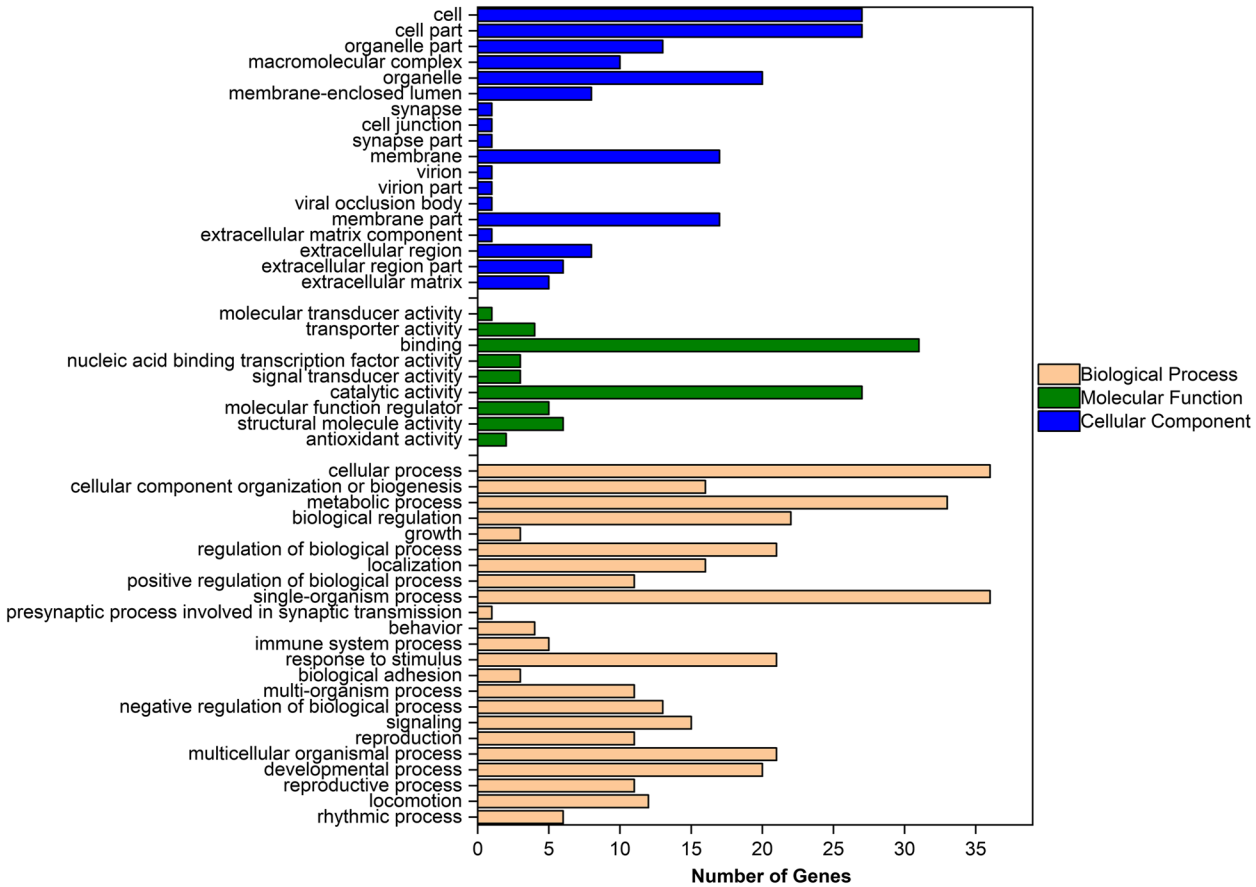


Fig. 5 GO term enrichment analysis of target genes of DEMs. The three main categories of Gene Ontology (GO) are biological process, cellular component, and molecular function. The x-axis represents the count of genes, whereas the y-axis denotes the GO terms at the second level of hierarchy

Discussion

The DBM is a globally recognized pest that inflicts damage on cruciferous crops. This pest has developed resistance to various pesticides commonly employed for its control [2]. As an alternative to chemical measures, biocontrol strategies utilizing fungus-based agents have gained attention [5, 38]. Previous research has highlighted the potential of the entomopathogenic fungus *I. fumosorosea* in effectively combating DBM [39, 40]. Therefore, comprehending the innate immune defense mechanisms of DBM would prove valuable in enhancing the biocontrol of this pest using entomopathogenic fungi. One study explored immunity-related genes in DBM treated with *I. fumosorosea* and identified defense-related DEGs in DBM against the fungus [5]. Another study emphasized the significance of conserved miRNAs in the interaction between DBM and *I. fumosorosea* [19]. These findings collectively establish the existence of immune-related mRNAs and miRNAs in DBM as a response to fungal infection. However, previous investigations have focused on examining miRNA and mRNA transcripts independently. Through our study, we conducted an integrative analysis of miRNA-mRNA interactions to provide a comprehensive understanding of the biological processes involving miRNA-mediated mRNA participation in DBM.

In this study, several immune-related genes were detected in DBM after exposed to *I. fumosorosea* and these genes were classified as immune recognition families, signal modulation, immune signaling pathways and immune effectors and others. Most of the immune-related genes exhibited up-regulated expression, while a subset displayed down-regulated expression. This finding is largely consistent with the extensive alterations in immune-related gene expression reported by Wang et al., [27], highlighting the dynamic balance between host immune responses and pathogen infection strategies. The up-regulated genes are typically associated with the host defenses. These genes likely encode proteins that are involved in recognition, signaling, and effector functions, which activate the innate immune system. For instance, the up-regulation of pattern recognition receptor (PRR)

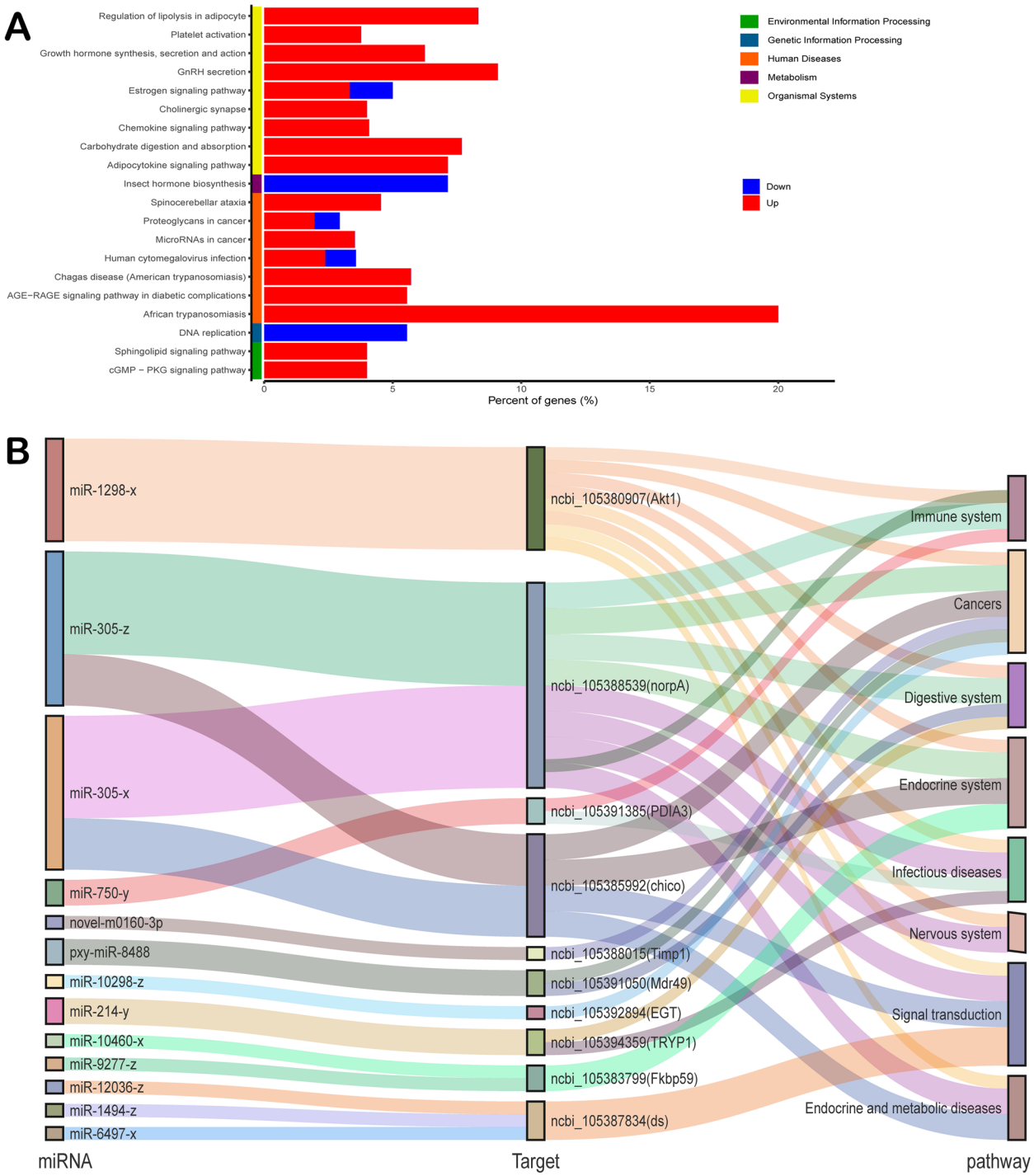


Fig. 6 **A** The top 20 KEGG pathways enriched of of target genes of DEMs (Level 3). **B** A Sankey diagram illustrates the network of miRNA-target-(immune-related pathways). Each rectangle signifies a miRNA, a gene, or a pathway, and the interconnections among the genes are depicted visually. Akt1, RAC serine/threonine-protein kinase; norpA, 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like; PDIA3, protein disulfide-isomerase A3; chico, uncharacterized protein LOC105385992; Timp1, metalloproteinase inhibitor 3; Mdr49, multidrug resistance protein homolog 49-like; EGT, UDP-glucuronosyltransferase; TRYP1, trypsin, alkaline C-like; Fkbp59, FK506-binding protein 59-like isoform X1; ds, protein dachsous

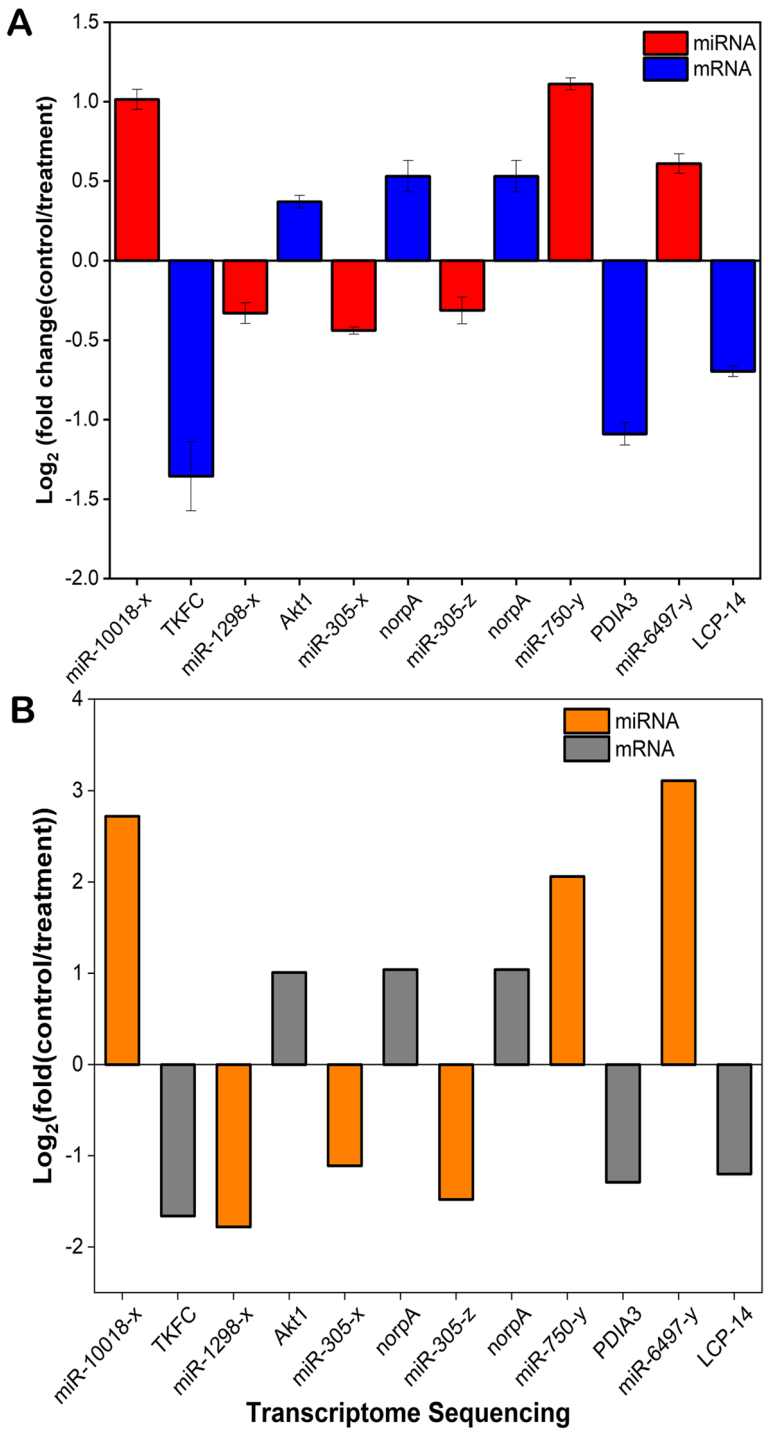


Fig. 7 Comparison of expression profiles of miRNA-mRNA pairs in *Plutella xylostella* upon fungal infection, as determined by quantitative real-time PCR and transcriptome sequencing. **A** Verification of differentially expressed miRNA-mRNA pairs using quantitative real-time PCR. Red boxes denote miRNAs, and blue boxes denote their corresponding target mRNAs. **B** Transcriptome sequencing results for the same miRNA-mRNA pairs. The orange boxes indicate miRNA and the gray boxes indicate their target mRNAs. MiR-10018-x targets TKFC (triokinase/FMN cyclase-like). miR-1298-x targets Akt1 (RAC serine/threonine-protein kinase); Both miR-305-x targets and miR-305-z target norpA (1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase-like); miR-750-y targets PDIA3 (protein disulfide-isomerase A3); miR-6497-y targets LCP-14 (adhesive plaque matrix protein-like isoform X1)

genes may enhance the recognition of fungal pathogens, while the up-regulation of effector genes, such as antimicrobial peptides and lysozyme, directly targets the fungal cell wall or membrane, resulting in damage to the pathogen or inhibition of its growth [41–43]. The down-regulated genes may indicate an adaptive strategy employed by the host in response to fungal infection, or they may result from the pathogen's suppression of the host immune response through various mechanisms [8].

Immune recognition of pathogen

The insect innate immunity is crucial for reducing infections by pathogens [44]. This innate immune response begins with microbial recognition, where host pattern recognition receptors (PRRs) detect conserved pathogen-associated molecular patterns (PAMPs) [45]. In insects, PRRs such as peptidoglycan recognition protein (PGRPs), β-1,3-glucan recognition proteins (βGRPs), and lectins play essential roles [46]. Previous investigations in DBM identified two up-regulated PGRPs at 24 h post-infection by *I. fumosorosea* [5]. Other studies have used RNAi methods to confirm the key role of PGRP in mosquito antifungal responses [41]. Our study identified two up-regulated PGRPs in DBM 24-h post-treatment with *I. fumosorosea*. The results of our study are similar to previous studies, indicating that fungal infection triggers the production and up-regulation of PGRPs in DBM. The other PRRs such as lectins, hemolin, and GNBPs were found to be downregulated in response to *I. fumosorosea* in DBM [5]. However, the expression of these PRRs was not observed to be induced in our study, which could be attributed to the use of different fungal strains and treatment duration.

Signal modulation

Serine proteases (SPs) are involved in various biological processes, including innate immunity [47]. In this study, we identified multiple serine protease family genes in DBM with elevated expression following a 24-h infection by *I. fumosorosea*. A similar increase in serine proteases was observed in DBM when exposed to *B. bassiana* [27]. Additionally, both up-regulated and down-regulated serine protease genes were detected at various time points following the infection of DBM by *I. fumosorosea* [5]. These findings indicate that fungal infection significantly influences the expression of serine protease family genes in DBM.

Insect metalloproteinase inhibitors could inhibit the activity of metalloproteinases that are secreted by pathogens. These metalloproteinases are responsible for degrading immune-related peptides in infected hosts [29]. A study employed RNAi technology to elucidate the role of metalloproteinase inhibitor genes in the resistance of *Galleria mellonella* larvae to infection by *Metarhizium anisopliae* [48]. Our study discovered a significant up-regulation of metalloproteinase inhibitors in DBM, suggesting that when confronted with *I. fumosorosea* infection, DBM may hinder the invasion of fungus by releasing metalloproteinase inhibitors.

Immune signaling pathways

The insect immune system is regulated by several signaling pathways, including Toll, IMD, and JAK/STAT [49, 50]. Activation of Toll and Imd signaling pathways is a typical immune response in fungus infected insects [51]. The Toll pathway is primarily activated by fungi and Gram-positive bacteria, whereas the IMD pathway is predominantly induced by Gram-negative bacteria [52]. Nevertheless, recent studies have demonstrated that treatment of *Monochamus alternatus* with *Metarhizium anisopliae* activates the Toll and IMD signaling pathways [53]. Our study found that infection of the DBM by *I. fumosorosea* also led to the activation of Toll and IMD signals, resulting in the upregulation of several immune-related genes and the production of antimicrobial peptides (AMPs). ModSP, a modular serine protease, plays a crucial role in activating the Toll pathway as a response to fungal and Gram-positive bacterial infections [30]. Previous studies have highlighted the significance of ModSP in the immune response of *Spodoptera exigua* against fungal infection [54]. Our study found a significant increase in ModSP gene expression in DBM after 24 h of fungal exposure, highlighting its potential key role in Toll pathway activation during fungal infections. Serine/threonine-protein kinase pelle-like (pll) is the sole *Drosophila* IRAK homolog associated with the conserved Toll pathway, which regulates innate immune responses [31]. This study showed significant up-regulation of pll expression, indicating its potential role in DBM innate immune response via the Toll pathway during fungal infection. The JAK/STAT pathways, similar to the Toll and IMD pathways, are important in the immune response to pathogens. Here, we identified a single RAC serine/threonine-protein kinase gene in the JAK/STAT pathway.

Immune effectors

Melanization, aided by the prophenoloxidase (PPO) enzyme cascade, is an effective insect defense against pathogens [55, 56]. Upon pathogen recognition, the prophenol oxidase-activating protease (PAP) enzyme can cleave inactive PPO, converting it into active phenol oxidase (PO) and ultimately leading to the eradication of the pathogen [32]. In this study, two PAPs were identified, and both exhibited up-regulation in DBM after treatment with *I. fumosorosea*. Previous research has demonstrated

that PAPs expression is induced when *Frankliniella occidentalis* are infected with *B. bassiana* [57]. These findings suggest that PAPs may play a crucial role in insect resistance against fungal infections.

Apolipoprotein-III plays a crucial role in lipid transport, lipoprotein metabolism, pathogen recognition, and the immune response in insects [58, 59].Our study found that the expression of apolipoprotein-III decreased in DBM after 24 h of exposure to *I. fumosorosea*. Previous studies have demonstrated that the expression of apolipoprotein-III decreases during the initial stage (12–24 h) of DBM treatment with *B. bassiana*, followed by a significant increase in expression 36 h after treatment [27]. These findings suggest that DBM may employ different innate immune strategies to combat fungal infections.

AMPs play an important role in host defense against microbial pathogens [60]. In this study, cecropin and gloverin were identified in DBM treated with *I. fumosorosea* for 24 h. The expression of all the AMPs, including four cecropin and one gloverin (Table 1) showed upregulated expression. However, the expression of these five AMPs in DBM was found to be downregulated in response to *I. fumosorosea* infection [5]. Additionally, the four cecropins were not detected in DBM 24 h after treatment [5]. These variations in results could be attributed to differences in fungal strains, sample size, and sample heterogeneity. Previous studies have demonstrated an increase in cecropin expression in DBM when exposed to *M. anisopliae* [61], as well as in *Spodoptera exigua* when exposed to *Metarhizium rileyi* [54]. Our findings align with these previous findings. The results of our study indicate that cecropins and gloverin may play a unique role in the innate immune response of DBM against *I. fumosorosea* infection.

Previous study found that GO terms such as response to stimulus, biological regulation, catalytic activity, binding, membrane, and biological regulation were significantly enriched in *I. fumosorosea* infected DBM [5]. In the present study, we observed that cellular process, single-organism process, cell part, cell, binding, and catalytic activity were enriched in *I. fumosorosea* infected DBM. These terms are closely associated with physiological and metabolic processes, suggesting that the immune response triggered by fungal infection induces notable physiological and metabolic alterations in the insect host [62, 63].

miRNAs are pivotal in controlling various biological processes in insects, including growth and development, immunity, antimicrobial defense [64, 65]. Previous studies have successfully identified a total of 170 miRNAs in *M. anisopliae* -infected DBM. Furthermore, this research revealed that conserved miRNAs, such as miR-281, miR-263, miR-1, miR-6094, and miR-8, exhibited significantly increased expression levels [66]. Similarly, a study on DBM treated with *I. fumosorosea* identified several conserved DEMs that play roles in host–pathogen interactions, including miR-2, miR-9a, miR-745, miR-7b, and miR-2767 [19]. In this study, we discovered that several conserved miRNAs, including miR-6497, miR-8488, miR-8515, and miR-8534, exhibited differential expression in DBM infected with *I. fumosorosea* for 24 h. These findings indicate that these miRNAs may have significant implications for DBM's immune response to *I. fumosorosea*.

Some highly abundantly expressed miRNAs were identified in this study, including miR-305, miR-6497, and miR-981. These miRNAs have been previously reported to play diverse roles in other insects. For instance, in *Aedes aegypti*, miR-305 directly targets the gene AAEL009899 and participates in regulating egg development [67]. In the flesh fly, *Sarcophaga bullata*, miR-305 may be associated with diapause [68]. Similarly, miR-305 in the Colorado potato beetle, *Leptinotarsa decemlineata*, may be linked to resistance against chlorantraniliprole [69]. Moreover, recent studies suggest that miR-305 may be involved in the interaction between *Bactrocera dorsalis* and the intestinal microbiota [16]. In our study, we observed that miR-305-x and miR-305-z regulate the most target genes, with 11 and 10 target genes respectively. In previous studies, miR-6497 in *Bivoltine Silkworm* was found to directly regulate bmcycle and subsequently regulate circadian rhythm genes, thereby contributing to the diapause induction process of the species [70]. Additionally, miR-981 has been reported to target the pdpc gene and participate in regulating the metamorphic development of the oriental fruit fly, *Bactrocera dorsalis* [71]. Therefore, it is possible that miR-305, miR-6497, and miR-981 are involved in the immune defense of DBM against fungal infection. However, further research is needed to understand their specific regulatory mechanisms.

Several DEMs identified in this study, including miR-279, miR-3533, miR-750, miR-965, miR-5119, and miR-1298 have also been reported in other species. Previous studies have observed the presence of miR-279 in DBM infected with detruxin A, a key virulence factor of the entomopathogenic fungus *M. anisopliae* [72]. Additionally, a high level of miR-279 expression was detected in DBM infected by *M. anisopliae*, suggesting its potential role in the immunity of DBM against fungal infection [63]. Similar to their findings, our study observed a significant up-regulation of miR-279 expression in infected DBM by *I. fumosorosea*, indicating its potential role in the antifungal response of the DBM. In Thrips, miR-3533 may be involved in larval and adult development, metamorphosis, parthenogenesis, and reproduction [73]. Studies have demonstrated that miR-3533 can enhance

the immune response of goat peripheral blood mononuclear cells (PBMCs) to the virulent Peste des petits ruminants virus (PPRV) [74]. Another gene, miR-750, may play a role in the resistance of Colorado potato beetle, *L. decemlineata* to spinosad [75]. Significantly up-regulated miR-750 regulates the expression of the sarcoplasmic calcium-binding protein (Scp), thereby promoting infection by the Penaeus monodon virus [76]. Furthermore, Huang et al. [77] demonstrated the interaction between miR-965 and silkworm fibroin L chain (Fib-L) in insect cells. Studies indicate that in shrimp, miR-965 facilitates the infection of the shrimp phagocytic virus by targeting the host ATG5 (autophagy-related 5) gene [78]. Previous research has shown that miR-5119 can target mRNA encoding various negative immune regulatory molecules, and thereby participating in anti-tumor cell responses [79]. Our analysis revealed that miR-5119 regulates six target genes, highlighting its potential significance in the regulatory network. Additionally, studies have indicated that miR-1298 can bind to the disintegrin metalloproteinase 9 domain (ADAM9) and effectively inhibit the development of breast cancer (BC) cells [80]. In mammals, miR-1298-3p targets the basement membrane protein Nidogen-1 (NID1) and plays a significant role in inhibiting tumor factors [81]. These findings suggest that miR-1298 may serve as a potential therapeutic target. Our study identified that miR-1298 as a potential regulator of multiple genes within the miRNA-mRNA network. Collectively, our findings suggest that miR-279, miR-3533, miR-750, miR-965, miR-5119, and miR-1298 may play crucial roles in DBM immunity to fungal infection, and their regulatory mechanisms require further investigation.

In the functional annotation analysis of DEM-DEG pairs, it was found that the immune system was enriched after fungus infection. Further analysis found that the four DEMs participated in the regulation of immune system pathways by affecting the expression of Akt1, norpA and PDIA3. These pathways encompass the Toll-like receptor signaling pathway, platelet activation, NOD-like receptor signaling pathway, T cell receptor signaling pathway, C-type lectin receptor signaling pathway, Fc epsilon RI signaling pathway, and Fc gamma R-mediated phagocytosis signaling pathway, etc. The Toll-like receptor signaling pathway is a common immune-related pathway, plays a crucial role in the host's response to pathogen infection in insect [82]. A recent study discovered that DEGs related to the Toll-like receptor signaling pathway could potentially contribute to the immune response of *Apis mellifera ligustica* towards *Ascosphaera apis* [83]. Platelet activation, another immune-related pathway, is a defense mechanism of arthropods against the host [84]. Furthermore, other immune-related pathways, such as the NOD-like receptor signaling pathway, T cell receptor signaling pathway, C-type lectin receptor, Fc epsilon RI signaling pathway, Fc gamma R-mediated phagocytosis signaling pathway, have been implicated in the immune response to viral or microbial infections in animals [36, 85–87]. This indicates that the above-mentioned miRNA-mRNA pairs play an important role in the immune defense of DBM against fungal infections. Additionally, the correlation analysis between DEMs and DEGs demonstrated significant enrichment in KEGG pathways associated with the endocrine system, signal transduction, digestive system, nervous system, and infectious diseases (Table S8B). This suggests that the regulation of these pathways by miRNA-mRNA pairs may serve as a defense mechanism against fungal infection in DBM.

In the study of insect immune responses, several investigations have explored the changes in expression levels of miRNA and mRNA, as well as their interactions. For instance, an integrative analysis of mRNA and miRNA expression profiles in indoxacarb-sensitive, resistant, and field-resistant strains of *Spodoptera litura* revealed negative correlations between 10 DEMs and 37 DEGs [88]. Zhang et al.[17] identified miRNAs and their target genes associated with cyantraniliprole tolerance in Asian sponge moth *Lymantria dispar*. Another study investigated alterations in miRNA and mRNA expression following the depletion of the gut microbiota in female *Bactrocera dorsalis*, highlighting the significant role of intestinal microbes in the gene regulatory networks of insects [16]. These studies provide valuable insights into the mechanisms of gene regulation in insects under external stress. However, research on the correlation between DEMs and DEGs in response to fungal infections in insect pests remains limited. Our study identified various DEMs and their target genes associated with the immune defense of DBM against fungal infections. Additionally, we employed RT-qPCR to validate six pairs of miRNA-mRNA interactions. The remaining identified miRNA-mRNA pairs require further verification, which will offer additional insights into the regulatory networks delineated in our study. Annotation of these target genes of DEMs revealed that they are primarily enriched in immune-related pathways, signal transduction, the digestive system, the endocrine system, and pathways related to infectious diseases, suggesting that miRNAs may play a role in the host's antifungal defense by regulating the expression of genes within these pathways. Furthermore, the study found that the miRNA target genes associated with *L. dispar* tolerance to cyantraniliprole are primarily enriched in functional modules, including oocyte meiosis, MAP kinase activity, drug metabolism—other enzymes, and ABC transporters, etc. This suggests that

miRNA may influence insect tolerance to pesticides by modulating gene expression within these functional modules [17]. In female *B. dorsalis* treated with antibiotics, the target genes were primarily annotated for processes such as energy metabolism, oogenesis, innate immune response, and growth and development, etc. This suggests that miRNA may mediate the interaction between the host and intestinal microbiota by influencing gene expression in these pathways [16]. In summary, under various stresses such as pesticides, antibiotics, and insecticidal fungi, insects may adopt different coping strategies. Moreover, miRNA may serve a significant regulatory function in various host stress responses; however, its regulatory mechanisms need further investigation.

Previous studies have reported low expression levels of new miRNAs in DBM infected with *M. anisopliae* [63]. Consistent with these findings, our study also observed relatively low expression levels of the new miRNAs in DBM infected with *I. fumosorosea*. Additionally, we predict a negative correlation between the new miRNAs and their target genes, indicating their potential involvement in the immune response of DBM against fungal infections. The miRNAs negatively correlated with target genes identified in this study have not been previously reported to play a role in insect resistance or immune responses to other biotic or abiotic stresses. Our findings open new avenues for research into the post-transcriptional responses of insects to pathogens and significantly enhance our understanding of the molecular mechanisms underpinning insect immunity against fungal infections. To the best of our knowledge, this is the first report of a miRNA-mRNA regulatory network in insects that responds to fungal infection. These findings highlight the critical roles of miRNAs in fungal infection and host immunity. However, additional experimental validation is necessary to elucidate the immune molecular mechanisms linked with these miRNAs. Moreover, the collection and analysis of data at additional time points of IFCF-infected DBM are necessary to enhance our understanding of the gene expression dynamics that occur during interactions between the fungus and the pest.

Conclusions

In summary, our study identified 580 DEGs and 55 DEMs in DBM infected with *I. fumosorosea*. We found 28 DEGs related to DBM immunity to *I. fumosorosea* infection. Through a comprehensive correlation analysis of mRNA-seq and miRNA-seq data, we identified 87 negative correlation pairs between miRNA and mRNA in *I. fumosorosea*-infected DBM. Among these, 13 miRNAs and their corresponding 10 mRNAs were identified as candidate miRNA-mRNA pairs associated with DBM immunity against fungal infections. Our study suggests that miRNAs may play a role in the host antifungal immune response by regulating gene expression across various pathways. In summary, the comprehensive analysis of miRNAs and their target genes in *I. fumosorosea*-infected DBM lays a foundation for further research on the roles of miRNAs and their targets in the immune defense of DBM against fungal infections. This research not only deepens our understanding of insect immune responses to fungal infections but also offers a valuable resource for the development of new insecticides.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-11192-3.

Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions

M.Q. contributed to the study's conception and design. M.Q. and L.J. conducted the experiments. M.Q., L.J. and H.M. analyzed the data. M.Q. and L.J. wrote the first draft of the manuscript. S.J. and M.Q. revised the manuscript and interpreted the data. All authors read and approved the manuscript.

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Data availability

The RNA-Seq data were submitted to Sequence Read Archive (SRA) database under accession number (SRR27194052; SRR27194051; SRR27194050; SRR27194049; SRR27194048; SRR27194047; SRR27166045; SRR27166044; SRR27166043; SRR27166042; SRR27166041; SRR27166040). The mRNA datasets and sRNA sequencing have been deposited in the National Center for Biotechnology Information SRA with accession numbers PRJNA1050803 and PRJNA1051778, respectively. The other data that support the findings of this study are provided in Supplemental information.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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