Viral derived miRNAs in OrNV-*Oryctes rhinoceros* interaction

Kumar Arvind[1], Ginny Antony[2], Rajesh M.K.[3], Josephrajkumar A.[4] & Tony Grace[1]*

[1]*Department of Genomic Science, Central University of Kerala, Kasaragod 671320, Kerala, India*

[2]*Department of Plant Science, Central University of Kerala, Kasaragod 671320, Kerala, India*

[3]*ICAR-Central Plantation Crops Research Institute, Kasaragod 671124, Kerala, India*

[4]*ICAR-Central Plantation Crops Research Institute, Regional Station, Kayamkulam 690533, India*

*Corresponding author. Dr. Tony Grace, Assistant Professor, Department of Genomic Science, School of Biological Sciences, Central University of Kerala, Tejaswini Hills, Periya, Kasaragod 671320, Kerala, India. Email-Id: tonygrace@cukerala.ac.in

Research Highlights

- Identified putative miRNAs from OrNV genome and their cognate gene targets in OrNV and *Oryctes rhinoceros* by insilico analysis.
- Viral derived miRNAs in OrNV infected beetle larvae were validated by qRT-PCR.
- The expression of one target gene elongation factor 2 (*EF2*) is downregulated in OrNV-infected larvae.

Abstract

Oryctes rhinoceros nudivirus (OrNV) is a double-stranded DNA virus known to cause lethal infections to the larval and adult stages of *Oryctes rhinoceros*, the rhinoceros beetle, a major pest of coconut and oil palm. However, in recent times, some beetle populations have been reported to develop resistance against the virus, affecting the efficacy of the OrNV as a biocontrol agent. Thus, there is an urgent need to elucidate the underlying mechanism of viral resistance in the host insect. Among the diverse group of small RNAs, microRNAs (miRNAs) are single-stranded endogenous non-coding RNAs that regulate myriad biological processes, including host-pathogen interactions. Viral encoded miRNAs are reported

to modulate host gene expression to enhance viral infectivity. In this study, we report the identification of OrNV-deduced miRNAs employing a genome-wide screening strategy and also predict their targets genes in the host *O. rhinoceros*. We have identified 21 miRNAs, which included novel as well as conserved and over hundreds of target genes utilizing transcriptome data of *O. rhinoceros*. We have also identified 14 potential target genes in the OrNV genome. These include genes involved in replication, protein ubiquitination, DNA binding, serine-type endopeptidase activity, and protein dimerization. The predicted viral derived miRNAs were detected in OrNV-infected larvae using qRT-PCR, and the reduced expression of one of the miRNA target *EF2* coincided with the detection of cognate miRNAs. We also discuss the possible mechanisms which could compromise the efficacy of OrNV against *O. rhinoceros* and the potential implications to pest management in general.

Keywords:

Oryctes rhinoceros nudivirus (OrNV), coconut, miRNA, target, gene, annotation.

1. Introduction

Isolated in the 1960s from *Oryctes rhinoceros* (The Coconut Rhinoceros Beetle) in Malaysia, Oryctes rhinoceros nudivirus (OrNV) possess a double-stranded DNA, enveloped by a rod-shaped virion of about 200–235 nm in length and 100–120 nm in width (Lee et al., 2015). The damage caused by *Oryctes rhinoceros* poses a severe threat to the livelihood of the farmers in the tropical region where coconut is a cash crop and considered as the tree of life (Etebari et al., 2020). The adult life stage of the CRB causes severe damage to the coconut by boring into the crown and feeding on the sap (Bedford, 2018, 2013). Both adults and larvae are the susceptible stages of the *O. rhinoceros* life-cycle, prone to infection by OrNV on feeding contaminated organic matter. Following feeding, OrNV penetrates the gut epithelium, finds its way into the hemocoel, and causes chronic infection to the fat body cells (Chaudhary et al., 2019). Besides, OrNV is reported to infect other members of the genus *Oryctes* viz., *Oryctes monoceros* (Bedford, 2013). During the 1960s and 1970s, OrNV emerged as a biocontrol agent

that curbed the invasive phase of the beetle (Bedford, 2018; Huger, 2005). The use of Oryctes rhinoceros nudivirus, OrNV, provided great relief to the farmers in the Pacific Islands for 30 years after implementing the biocontrol programme. The absence of CRB invasion after OrNV introduction in Pacific island asserts its effectiveness in the pest management strategy (Marshall et al., 2017). Reports of re-emergence of the rhinoceros beetle in these regions suggest the spread of a low virulence isolate of OrNV or the emergence of more tolerant beetle haplotypes (Etebari et al., 2020; Marshall et al., 2017). Exploring the molecular mechanism underlying the evolution of resistance in the population of CRB against OrNV could be a helpful step towards regaining its effectiveness.

Plants and other eukaryotes protect their genomes against aberrant nucleic acids through RNA silencing, a conserved defense mechanism. This process utilizes short RNAs (20-30nt) to identify and neutralize the complementary nucleic acids. Among the five well-known classes of the characterized small regulatory RNAs viz., microRNAs (miRNAs), small interference RNAs (siRNAs), transacting siRNAs (ta-siRNAs), natural antisense siRNAs (nat-siRNAs) and, in metazoans, the Piwi-interacting RNAs, miRNAs (miRNAs) have been observed to have a direct role in viral defense. Canonically, guide strand of miRNAs possessing seed sequence (complementary 2-7 bps region) target the mRNAs and causes their degradation or translational blockade (O'Brien et al., 2018). Deploying specific virulence factors, referred to as viral suppressors of RNA silencing (VSRs), viruses tend to manipulate the host miRNA and exploit them to serve their purpose, viz., replication and propagation (Bruscella et al., 2017).

Many viruses are also found to encode miRNAs (Teng et al., 2015). Viral miRNAs within the host cell may help establish a cellular environment conducive to their replication by mimicking the conserved gene regulatory mechanism (Bruscella et al., 2017; Skalsky and Cullen, 2010). In many virus-host interaction systems, viral-derived miRNAs regulate viral gene expression

and help in the evasion of immune responses of the host (Mishra et al., 2020). Bombyx mori nucleopolyhedrovirus(BmNPV) miR-3 regulates the expression of P6.9, a gene important for infectious virion formation (Singh et al., 2014). It is proposed that the regulation of late expressing genes in the early stages of infection minimizes viral load and thus helps the virus evade the immune response of the host. Viral miRNAs targeting host genes have also been identified and may act as regulators of host defense genes and help create a favorable intracellular milieu (Mishra et al., 2020). The endoparasitoid of diamond black moth *Plutella xylostella*, *Cotesia vestalis*, and its associated Bracovirus (CvBV) produce different miRNAs that regulate the same target in the host (Wang et al., 2021). Heliothis zea nudivirus1(HzNV-1) is a DNA virus that infects several insects and employs miRNAs to promote lytic infection (Wu et al., 2018).

Entomopathogenic viruses, in many instances, have been developed as potent bio-insecticide with success, but the knowledge about the molecular mechanism underlying the pathogenesis is rudimentary. The complete genome of the OrNV is ~127 kb with 130 ORFs (Etebari et al., 2020; Lee et al., 2015). In a recent study in OrNV, Etebari et al. (2020) reported a miRNA, i.e., OrNV-miRNA-1, as the single most abundant sRNA sequence among the OrNV-derived small RNAs. In this study, we screened the genome of OrNV in a bidirectional manner and identified 21 miRNAs. The potential miRNAs target genes were identified using the transcriptome sequence of *Oryctes rhinoceros* from our previous study (Arvind et al., 2020). The expression level of candidate miRNAs was evaluated in the OrNV infected larvae seven days' post-treatment. We also analysed the expression of one of the putative targets, i.e., *EF2*, in the infected larvae.

2.1 Material and methods

2.2 Retrieval of OrNV genome sequence data and screening

Complete genome sequence of Oryctes rhinoceros nudivirus (OrNV) genome (Accession number NC_011588.1) was retrieved from the NCBI Genome database. The VMir Analyzer was used to identify miRNA hairpins encoded by the OrNV genome (Grundhoff and Sullivan, 2011). Considering OrNV being a circular double-stranded genome sequence, all hairpins in direct or reverse orientation for conformation and orientation were displayed. To discern the main hairpins, each identified sequence was further filtered using VMir Viewer (Grundhoff and Sullivan, 2011). The filter values for ‘‘minimal score’’ and ‘‘window counts’’ were set to the most stringent parameters of 115 and 35, respectively, as suggested previously (Teng et al., 2015).

2.3 Classification of Hairpins and Prediction of Secondary Structures

All putative OrNV pre-miRNA were screened using the standalone MiPred program to identify the secondary structure as suggested by Teng and the team (Teng et al., 2015). By virtue of MiPred, (Jiang et al., 2007), we distinguished the real from -pseudo-pre-miRNA, using a hybrid feature, including local contiguous structure sequence composition, Minimum of Free Energy (MFE) secondary structure, and p-value of randomization test. Pre-miRNA sequences with prediction confidence equal to or greater than 70% were considered for the downstream analysis. Subsequently, Mature Bayes, a Naive Bayes Classifier (NBC) (Gkirtzou et al., 2010), was utilized for deducing mature sequences of all pre-miRNAs.

2.4 Target prediction, annotation and network analysis

To predict miRNA targets, transcriptome sequences of *Oryctes rhinoceros* from our previous study (Arvind et al., 2020) and genome sequence of OrNV (Accession number NC_011588.1) were taken into account. Target genes for OrNV deduced mature miRNA was searched using

miRanda (Enright et al., 2003). For target annotation, mature miRNA sequences were searched for homology against the UniProt-SwissProt database (Bairoch et al., 2005) using standalone BLASTX (Altschul et al., 1990). Using customized parameters viz., percent identity ≥ 80% as well as bitscore ≥ 50, the blasts hits were filtered and considered for downstream analysis. Gene enrichment and COG pathway analysis were performed using DAVID (Dennis et al., 2003). We also analysed the interaction between mature miRNAs and their potential targets employing Cytoscape 3.8.2 (Shannon et al., 2003).

2.5 Larvae infection with OrNV

The larva of *O. rhinoceros* was reared in our lab facility following standard method at 25°C at 25–30 °C with a 16-h light/8-h dark photoperiod. They were kept temporarily for 2 to 3 days and acclimatized prior to the experiments. CPCRI OrNV-701 isolate (Kerala, India) was used for this study. The virus-free larvae were infected with OrNV following the previous study (Marshall et al., 2017) and regularly monitored for physiological changes and mortality for nine days. For comparison, the control group larvae without OrNV treatment were also reared, maintained, and regularly monitored for the given time duration.

2.6 Extraction of miRNA

Post eight days of OrNV treatment, total RNAs were extracted from the infected gut (of both OrNV treated and non-treated subjects using a mirVana RNA isolation kit (Ambion) following the manufacturer's instructions. To measure the purity and concentration of extracted miRNA, an ultraviolet spectrophotometer (Nano-Drop-2000, Thermo Scientific) was used. The quality and the purity of the extracted RNA were assessed by OD 260/280 ratio, and RNA integrity number (RIN) was analysed using an Agilent Technologies 2100 Bioanalyzer with the Agilent RNA chip with RIN value ranges from 9.3 – 9.6. Subsequently, the high-quality RNA was

reverse transcribed to cDNA using miScript Reverse Transcription Kit according to the manufacturer's instructions (Qiagen). Given the fact that endogenous miRNAs are not polyadenylated, during the reverse transcription process, a poly(A) polymerase polyadenylates the miRNA. Following which miRNA works as template to synthesize cDNA using oligo-dT and Reverse Transcriptase.

2.7 Primer designing and Quantitative real-time PCR

All the forward primers designed for the selected miRNAs are based on the mature miRNA sequences (Table 1). Reverse universal primer provided with the miScript II RT kit was used commonly for all reactions. The list of 8 forward sequence primers is given in table 1.

To examine the expression level of potential candidate miRNAs in the OrNV-treated and the control group, real-time PCR was conducted. Each qRT-PCR reaction was conducted in a volume of 10 µl containing 5 µl of SYBR green, 5 pmol of forward and reverse primers, and 1 µl of cDNA (400 ng). For the reaction run, three biological replicates with three technical replicates of the treatments were performed. The non-coding small nuclear U6 RNA was used as a reference gene (Huang et al., 2014). The PCR protocol was 94°C for 5 min followed by 40 cycles of 94°C for 40 s, 60°C for 30 s, and 72°C for 45 s, with a final elongation at 72°C for 10 min. RT-qPCR data output was normalized to that of U6 snRNA using the $2^{-\Delta Ct}$ method and was analysed using the $2^{-\Delta\Delta Ct}$ method following a previous study (Wang et al., 2018). One-way ANOVA was used to determine the difference between the two groups. p-value < 0.05 was considered significant using R.

	MiRNA Id	**Forward primers (5'-3')**
1.	MD29	GGCCTTCAACACTTCGG

2.	MR539	GGACGGTGTTTAGGCAGC
3.	MR957	GGCGGCTTTGATTATTGA
4.	MD1025	GCCGGGCAACATATATGT
5.	MD1200	TGGCATCGACGCTCAG
6.	MR1292	CGGCCTAATTCTGGAGC
7.	MR1353	CGTGCAATTGTTGGAGG
8.	MD1366	GCGCGATGTATACGTCG
9.	*EF2* (Forward)	FGGTACGCTGGATAGCCTTGA
	EF2 (Reverse)	CCTTCGGTCGTGTCTTCTCT

Table 1: List of real-time PCR primers for candidate miRNAs and the target gene.

3. Results

3.1 Identification of OrNV derived miRNA

Following the screening of the OrNV genome, 21 real microRNA precursor candidates were identified. Details of the novel and conserved miRNAs can be found in Tables 2 and 3. Using MiPred, the integrity of pre-miRNA structures was confirmed, and also they were classified into real or pseudo-pre-miRNAs. We analysed the potential stem-loop structures of pre-miRNAs for the total number of base pairing in the stem region, GC pairs in the stem region, GU pairs in the stem region, the total number of Loops in the structure, total number of bulges in the structure as well as the free energy of the secondary structure.

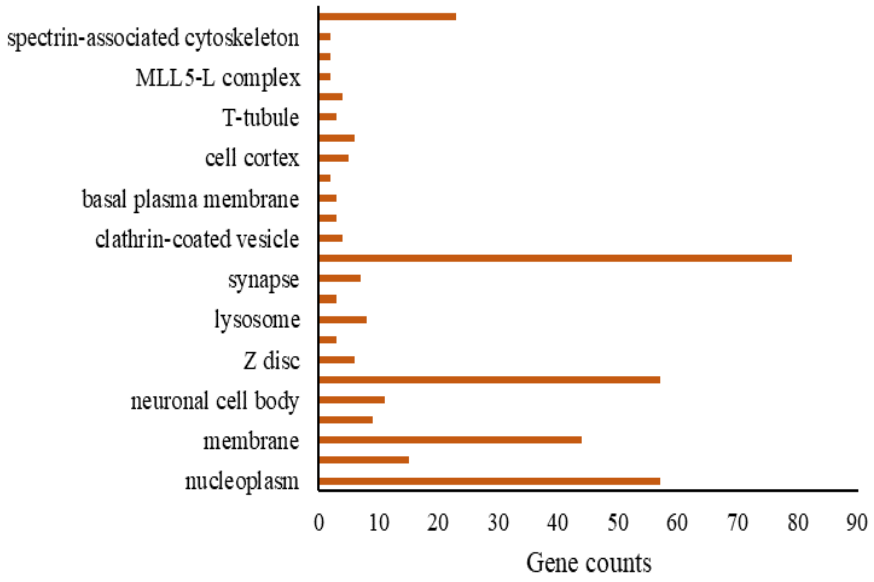
Based on the homology against the miRBase database, 14 potential pre-miRNAs were identified to be conserved among different related organisms. Among the conserved miRNAs, MD235 and MD1025 were found to be highly conserved. While MD235's mature regions represent homology with seven different organisms, MD1025's mature regions were found to be homologous with 25 different organisms. In addition, we also identified seven potential novel miRNAs viz., MR29, MD407, MR539, MR957, MD1200, MR1353, MD1366), and these were found to have no match with any sequence present in the miRBase database.

3.2 Target genes of OrNV deduced miRNA

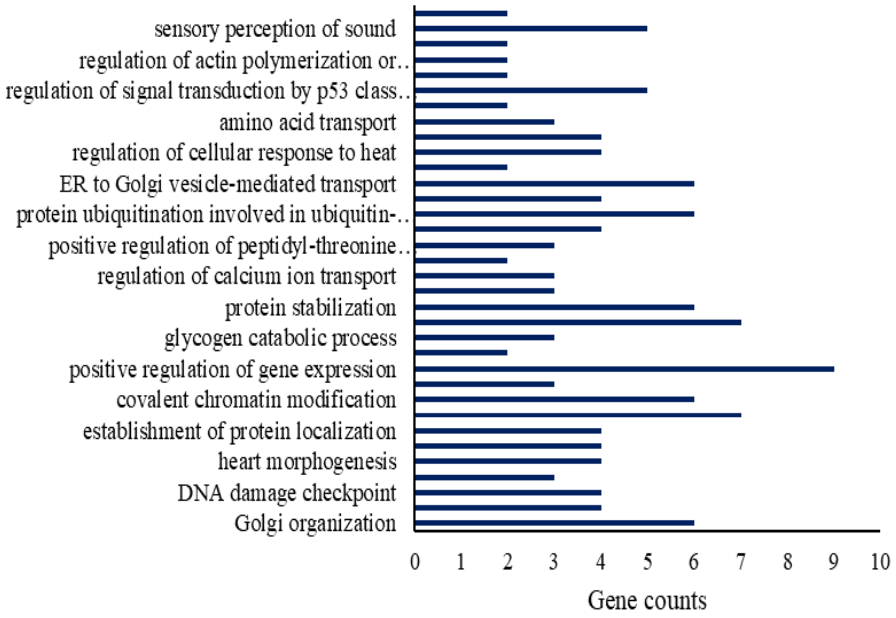
The putative targets of the miRNAs were predicted against the total 3' UTR region extracted from the transcriptomes of *O. rhinoceros*. The putative target functions were confirmed using the Uniprot database, following which gene ontology (GO)was performed (Figure 1). Following stringent parameters, we discerned more than a hundred potential targets from the transcriptome, and these include genes coding for; histone four protein, Sodium/potassium-transporting ATPase subunit alpha, Vacuolar-ATPase, calmodulin (CaM), exonucleases, Tip attachment protein J, Cytochrome 450, Ubiquitin-conjugating enzyme, TATA-box binding protein, ATPase subunit beta, H3-K79-HMTase, Elongation factor 2 (*EF2*), and Translation initiation factor IF-2. Ontology-based annotation (Biological process terms) classified targets genes as mainly involved in transcription regulation, translation, DNA methylation, nuclease activity, and defense responses.

Based on the hybridization of miRNAs and regions in the whole genome of OrNV, 97 potential genes targets were identified. Out of which, only 14 mRNAs could get annotated using the Unioprot database. The OrNV genome targets included DNA polymerase 060R, Protein p74, Apoptosis inhibitor IAP, Capsid-associated protein AC83, DNA ligase, Ribonucleoside-diphosphate reductase large chain, Ribonucleoside-diphosphate reductase small chain A,

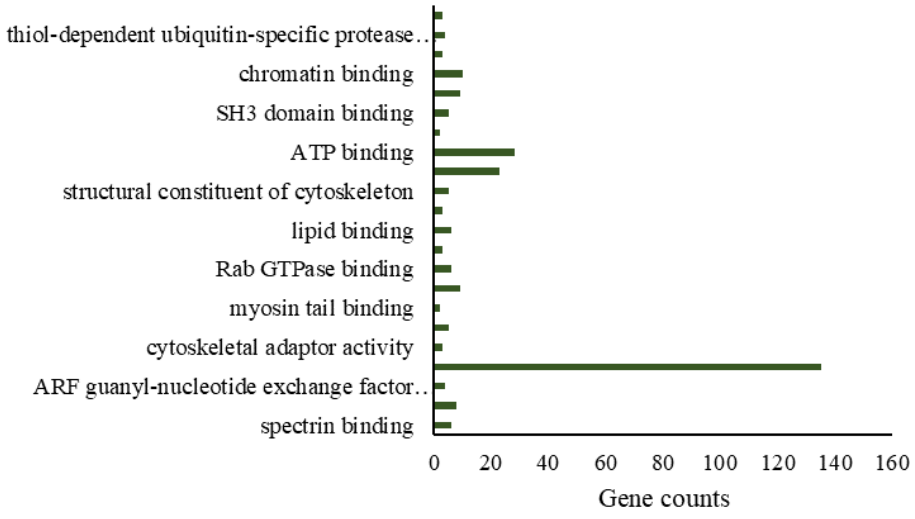
Semaphorin-1A, Serine protease 3, UPF0046 protein K07C11.7, Protein adenylyltransferase NmFic, Semaphorin-1A, Per os infectivity factor 1, and Thymidylate synthase. To comprehend the interaction between OrNV deduced candidate miRNAs with their identified mRNA targets, we sought to establish their interaction using Cytoscape. Multiple transcripts are revealed to be targeted by single miRNAs (Figure 2). Following the gene ontology results of OrNV miRNAs targets, we have also performed the clustering of orthologous genes (COG). The targets genes are clustered into two categories (a) chromatin structure and dynamics (b) cell division and chromosome partitioning (Figure 3).



(a)



(b)



(c)

Figure 1: Gene Ontology (GO) of miRNAs targets: (a) Biological processes (b) Cellular components (c) Molecular functions.

Ids	Sequence	length	Genomic location	MFE Value (Kcal/ mol)	Prediction confidence
MD29	GAUGCCGACCAUAUUAUUCCGGAUCACUAUUCAACACUUCGGUGUGAAUUGGUUCGGUGAAAAUUUGGUCGGCAUC	76	3781-3856	-37.60	76.7%
MD31	GUGAUGCCGACCAAAUUUUCACCGAACCAAUUCACACCGAAGUGUUGAAUAGUGAUCCGGAAUAAUAUGGUCGGCAUCGC	80	3779-3858	-32.50	79.3%
MR102	UCGAUGGCCGAUUCGCCGUCCAAUUGUGGAAGCCUAUACUGCUGCAUAUGGUCGACCGCACAUAUCAGGAUCCAUAAAAUGGCCGGCGAACGUGUUAGACUCGA	104	9670-9773	-34.70	74.5%
MD235	UUUGCACGAUUGCCUUCUAUGAUUGUGGUGUUUAAAUCGUUGAUCGCGUUCAAACACCACGGUAGAGCAUCGUUUGUAAA	80	22296-22375	-25.10	70.6%
MR330	CGUGCUAUACGGUGGAGUUUCGAACGGGGGAGUGAUUCUUGGGCGUUUACGUUUUGGUUCGGUCUGCUGUUGCUGCUGCUGUUGUUGUACG	91	30373-30463	-31.00	71.9%
MD407	CCGCCCAAGCUCCUACCGCGACCACAUCGAUGCUCGUCGGCGAAGCAGAGUCUGUAACCAUCCCGAUGAGUAUCGUUAUGGCGCGUGAAAGCGAGAACGG	100	48324-48423	-40.20	70.3%
MR539	GUACGAUCGUCAGCGUAUUGUUGAUUUGUUUAGGCAGCGUGUAAUAAAUGGCGGUCUUCAACUUCAAGUAAUACGUGUCCAUAAUUAAAUUGAGUAUAUUCUGACGACUGAGC	113	48580-48692	-27.80	74.5%
MD539	CCGAAAAGUACAUAAUAUGUGCUCUGAUUGAAUUGUCCGAAUUACUGAACGACAAGUCAUACUGCAAACUUGCGUUCGAAGUGAAUUCGUCCAGAUACGUUCAAUUGGCGCAAAAGUAUCUCGAAUCGG	129	51011-51139	-33.80	73%

MR593	GAAUAUAUGCAAAUCGCGACGAUAGUCUUCAUCG UGGAACCCUUUUUAUUUGAUAUAAAUAGACUAUA UAUUGUUGCGAUUUAGCAUUACUUC	93	53523-53615	-25.20	74.1%
MR711	GGUGGUUCUUGAUUUUAAACGCGUGAUUUCGCUC GAGAAUGCUGAGAAGCGUUACGUUUACGCCAAUU AUGCGUUUAAGGUUGUGCACCACU	92	66224-66315	-34.30	87.8%
MD783	GCUUUAAUAUUGAAAAAUAGCGUAUAAUGUAAAC UUAUAAUGUAAACUUAUAAUGCAAACUUAUAAUG UAAACUUAUAAGCGUACUGUUGUAUGUAGUUUUU UUAUUGC	109	70816-70924	-21.80	70.3%
MR906	ACGAUACGCUAAAAUUUGUCCGCCAAUUACGCGG UCUCGUAAAGUCGACCGUGUAAUUACGGACGACA CAGUUUGGUCUACGU	83	82649-82731	-30.30	73.6%
MD949	GUUUGUUUGUUAGAAAUUGAGGUUGAAUUUUGU CCGUCGACGCGCAACGUUCGACUAUUUGGUUGGG UUGGAUUUUGUCCGUCGACGAGCAACGUUUAGCC AUUUUCAACAUAUACAC	118	86166-86283	-43.20	83.8%
MR957	GCGAUGCCAUUUCUAACAUUUGAUUAUUGAAAAA CGGGUGUUUUUACAAAACAAGCCUGUUUUUCGAA AAUAUGUCAAUUUUUGUAUUAUAUGGCACGC	99	86994-87092	-29.20	82.7%
MD1025	GUAUGACAAAAAUGCAAUGUAAGGUUGAAGGUUG AAAGGUUGGAAAAAAAUACAACAUAUAUGUAUAU GUAUAUAUUUAUUGGUCUAACUCUUUCGCGGCCG UUUAACCAUACUCAUCAUGC	122	93953-94074	-29.70	72.4%
MR1072	UUUAAACGGUAUGCGCAAAUUGGCUGCCACUUGU GACAGUUGAAACCGUCCAAGUCCACGCACAUGCG CAUAAACGUAUAAA	82	97834-97915	-25.20	70.4%
MD1200	ACGGACCAGAGUUUUUAGAUCGACGCUCAGACUU UAUUAUGGCAUUGGUGUUGAUGAAGUCGUUGGGU GUUAUGAAUUUCUUGUAUGU	88	108013-108100	-22.50	73.3%
MR1247	CGAUUAAUCAAAAUGACCGACAAUUUCGUUGUGG AUUUGGUGUUUGUGAAACGGUUACUACUUCGACU	111	112086-112196	-26.20	72.6%

	GCAAGAUCGAUUCCACAGCAAGACUUGUUCAAUU UAGAUUUUG				
MR1292	UCCAUGUCGUAUUUGGUGCCGUCUUGAAGAUUUA UUUCCGGGCCCUUGUUAAUUCUGGAGCCUUCGGA AUUUACGACUAGCGCGUCCAAGUAAAUCAUGCGG UCGAUUAAACUCGAUAAUUGGA	124	115218-115341	-36.10	88%
MR1353	GAGCGUUGAAGUCGGGCGGCAUGUACGAAUUGUU GGAGGUUGCGAUCGUACGUACAUAGGUAGAUGAA UCGAUCGCAAUGUCCGUACGCUACGCAUAUCGUC UUAUAGUCGGCCAUUUCGUAGCUC	126	120688-120813	-46.70	76.3%
MD1366	UUGAUUAGUGUAUGUGGGAUAUGUAUACGUCGUA GAUAUUAUACGUAUUAAGCCGUAACUUUAUCUAA UGAUGGAUAUAUUCCGGGCUUAUUUGUUGA	98	121055-121152	-22.90	72.5%

Table 2: Details of the predicted precursor miRNA (pre-miRNA) in OrNV entailing Ids, sequence, length, genomic location, MFE (Minimum free energy) value, and prediction confidence.

OrNV miRNA Id	**5' Mature region**	**3' Mature region**	**Matches with 5' Mature region**	**Matches 3' Mature region**	**Type**
MD29	UCAACACUUCG GUGUGAAUUGG	AUUGGUUCGGU GAAAAUUUGGU	No match	No match	Novel
MD31	CAAUUCACACC GAAGUGUUGAA	AAUAGUGAUCC GGAAUAAUAUG	No match	bdi-miR7714-5p	Conserved
MR102	AAUUGUGGAAG CCUAUACUGCU	ACCGCACAUAUC AGGAUCCAUA	No match	dps-miR-2570-5p	Conserved
MD235	UUCUAUGAUUG UGGUGUUUAAA	AAACACCACGGU AGAGCAUCGU	dme-miR-9372-3p, mle-miR-2d-5p, gga-miR-16-2-3p, cpi-miR-16b-3p, ami-miR-16a-2-3p, cli-miR-16a-2-3p, pbv-miR-16b-3p, xla-miR-16b-3p	No match	Conserved

MR330	GGGAGUGAUUC UUGGGCGUUUA	UGGUUCGGUCU GCUGUUGCUGC	hpo-miR-10041-5p, dme-miR-1001-5p, dsi-miR-1001-5p	No match	Conserved
MD507	AUGCUCGUCGG CGAAGCAGAGU	CAUCCCGAUGAG UAUCGUUAUG	No match	No match	Novel
MR539	UGUUUAGGCAG CGUGUAAUAAA	UCAAGUAAUAC GUGUCCAUAAU	No match	No match	Novel
MD539	UUGUCCGAAUU ACUGAACGACA	UUGCGUUCGAA GUGAAUUCGUC	ppc-miR-8358-3p	No match	Conserved
MR593	AUCGCGACGAU AGUCUUCAUCG	CUAUAUAUUGU UGCGAUUUAGC	aae-miR-278-5p	No match	Conserved
MR711	UCUUGAUUUUA AACGCGUGAUU	AAUUAUGCGUU UAAGGUUGUGC	Egr-miR-4990, bmo-miR-3427	aly-miR395f-5p, aly-miR395h-5p	Conserved
MD783	UAGCGUAUAAU GUAAACUUAUA	UGUAAACUUAU AAGCGUACUGU	mdv1-miR-M12-5p	No match	Conserved
MR906	AAUUUGUCCGC CAAUUACGCGG	CCGUGUAAUUAC GGACGACACA	No match	ppt-miR1036-5p	Conserved
MD949	UCCGUCGACGC GCAACGUUCGA	UUGGAUUUUGU CCGUCGACGAG	No match	Gra-miR8650, mtr-miR5556-3p	Conserved
MR957	UUUGAUUAUUG AAAAACGGGUG	AAGCCUGUUUU UCGAAAAUAUG	No match	No match	Novel
MD1025	CAACAUAUAUG UAUAUGUAUAU	UAUAUAUUUAU UGGUCUAACUC	sme-miR-2173-3p, hsa-miR-1277-5p, rno-miR-466b-2-3p, rno-miR-466b-4-3p, tch-miR-1277-5p, cpo-miR-1277-5p, dno-miR-1277-5p, ocu-miR-1277-5p, mmu-miR-669f-3p, hsa-miR-1277-3p, ssc-miR-1277, ppy-miR-1277, hsa-miR-1277-5p, ggo-miR-1277, mml-miR-1277, bta-miR-1277, cpo-miR-1277-3p, dno-miR-1277-3p, ocu-miR-1277-3p, mmu-miR-669h-3p, mmu-miR-467g, hsa-miR-1277-5p, mmu-miR-297b-3p, mmu-miR-297a-3p, mmu-miR-297c-3p	oni-miR-10739	Conserved
MR1072	UGCGCAAAUUG GCUGCCACUUG	CGUCCAAGUCCA CGCACAUGCG	pca-miR-13b-5p	No match	Conserved

MD1200	AUCGACGCUCA GACUUUAUUAU	AUGAAGUCGUU GGGUGUUAUGA	No match		Novel
MR1247	AUCAAAAUGAC CGACAAUUUCG	GCAAGAUCGAU UCCACAGCAAG	No match	bfl-miR-10b, bbe-miR-10b-5p	Conserved
MR1292	UAAUUCUGGAG CCUUCGGAAUU	CCAAGUAAAUCA UGCGGUCGAU	stu-miR8033-3p,	No match	Conserved
MR1353	AAUUGUUGGAG GUUGCGAUCGU	UGAAUCGAUCGC AAUGUCCGUA	No match	No match	Novel
MD1366	AUGUAUACGUC GUAGAUAUUAU	ACUUUAUCUAA UGAUGGAUAUA	No match	No match	Novel

Table 3: Details of putative miRNAs identified from the genome sequence of Oryctes rhinoceros nudivirus (OrNV) and categorized as conserved and novel.

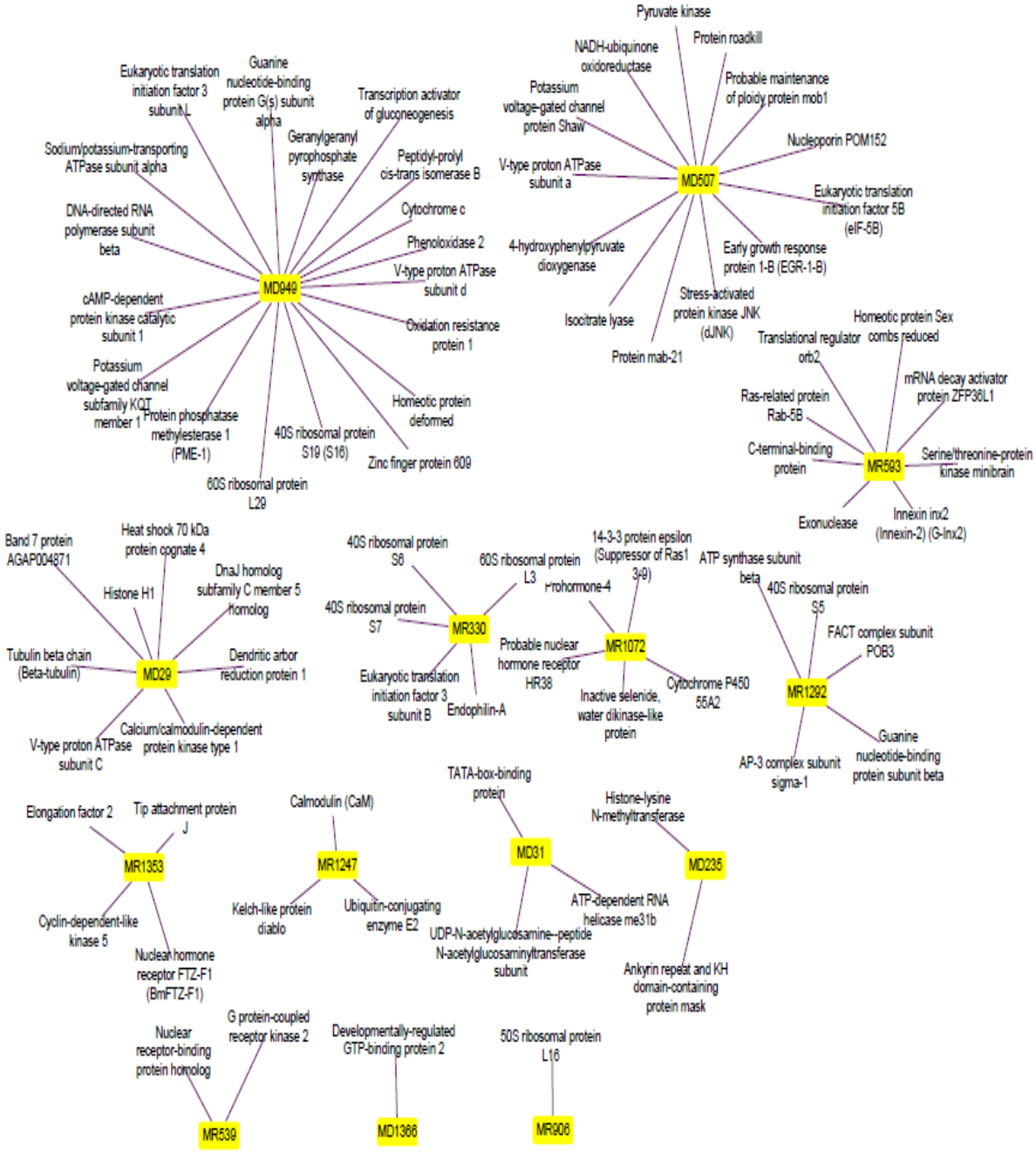


Figure 2: Certain OrNV deduced miRNAs and their mRNA targets. Owing to the interaction identified between miRNA seed region and 3'UTR region of the total transcripts from four developmental stages of *O. rhinoceros*, miRNA-mRNA network interaction is established.

3.3 Validation of miRNAs in OrNV infected CRB larvae

OrNV infected larva appeared beige, developed prolapsed rectum, and swollen gut tissues (supplementary material, Figure 1). To affirm the veracity of the Insilco results, eight candidate miRNAs viz., MD1200, MD1366, MR539, MD29, MR957, MD1025, MR1292, and MR1353 were selected for their expression analysis using qRT-PCR. The expression of all the candidate miRNAs was observed in the OrNV infected larvae (Figure 4a). Interestingly, the expression level of two of these (MD1200 and MD29) was comparatively higher. Also, the expression level of one of the crucial target genes of MD1025 and MR1353, i.e., Elongation factor 2 (*EF2*), was examined (Figure 4b). There was a significant decline in the expression level of *EF2* in the OrNV-treated larvae compared to the control (untreated larvae) group.

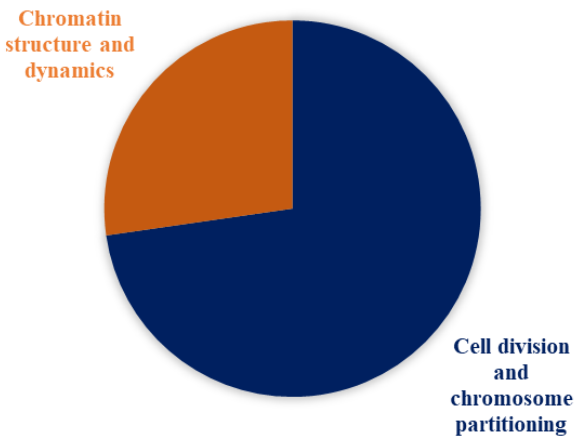
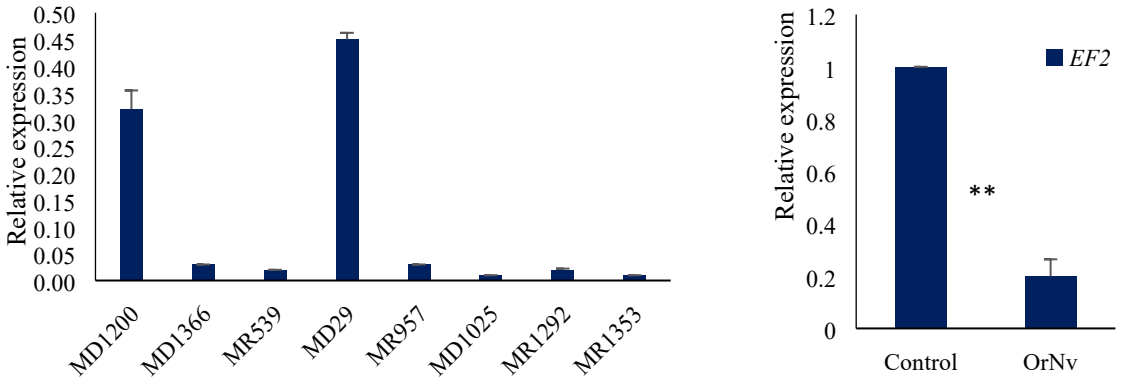


Figure 3: Cluster of Orthologous Genes (COG) based gene ontology of OrNV-deduced miRNAs targets.



(a) (b)

Figure 4: Expression level of (a) miRNAs identified in OrNV infected larvae. The U6 snRNA gene was used as an internal control. The expression levels of miRNAs were compared to the control (the non-infected group of larva). (b) *EF2* in OrNV infected larvae and control group. The expression of OrNV-encoded miRNAs was normalized to *O. rhinoceros* U6 snRNA. The statistically significant differences between them are represented with asterisks (*$p < 0.05$, **$p < 0.001$).

4. Discussion

Virus encoded miRNAs of entomopathogenic viruses are lesser-known entities. Only a few such miRNAs have been discovered (Etebari et al., 2020, and barring a few, their role in pathogenesis is still a mystery. Though for years, OrNV has been an effective biocontrol agent for coconut rhinoceros beetle (CRB) (Marshall et al., 2017), the molecular mechanism underlying its pathogenesis in the host remains unexplored. Recently, low-virulence isolates of OrNV from different geographical populations were examined for their genomic and transcriptional variations in chronically infected beetles (Etebari et al., 2020). In this study, we have identified 21 potential mature miRNAs derived from pre-miRNAs distributed at various genomic locations in the OrNV genome. While 7 of the 21 predicted miRNAs are found to be novel, 14 are observed to be conserved. Physiological changes such as developed prolapsed

rectum and swollen gut tissues observed in the OrNV-treated larval body were consistent with the previous study ((Lee et al., 2015). The evaluation of the expression level of miRNAs between control and the infected larvae indicated the deployment of miRNAs in causing infection and mortality. We analysed 8 out of the 21 miRNAs, and all were detected in infected larvae and not in non-infected larvae. Among them, MD1200 and MD29 were found to be highly expressed in all the infected larval subjects.

To gain insights into the host-pathogen interaction, the screening for miRNAs targets was performed using the transcriptome of *O. rhinoceros* (Arvind et al., 2020). Some of the potential targets of MD29 included DnaJ homolog subfamily C member 5 homolog, Glycine--tRNA ligase, Histone H1, NF1-X, Neurobeachin, Hsc 70-4, and V-type proton ATPase subunit C. DnaJ homolog subfamily C member 5 homolog, has a role in membrane trafficking and protein folding (Wang et al., 2004), was identified as one of the important miRNA targets in the Dengue virus (Ospina-Bedoya et al., 2014). Glycine--tRNA ligase is another predicted target of MD29, suggested as having a role in physiological RNA processing under conditions of cellular stress like virus infection. RNA ligases are utilized to maintain or manipulate RNA structure in response to purposeful RNA breakage events (Cai et al., 2020; Ho and Shuman, 2002; Tanaka and Shuman, 2011). During the DNA import mechanism of the virus into the host, the nuclear histone H1 assists in the transportation of DNA after viral capsid disassembly at the nuclear pore complex (Trotman et al., 2001). NF1 is a family of polypeptides that adheres to discrete DNA motifs and regulates gene expression in the cell (Nebl et al., 1994). Neurobeachin, another crucial target of MD1200, has been illustrated to have a role in electrical and chemical synapse formation as well as function to maintain the extensive dendritic branching in mature neurons (Miller et al., 2015). The interruption to the action of Neurobeachin has been revealed to cause a decline in the ability of an organism to elicit neural responses (Miller et al., 2015) and is likely a candidate for exploitation by OrNV to invade *O.*

rhinoceros. One of the most intriguing aspects of the interaction between viruses and the host is mediated by different heat shock proteins (HSPs) (Santoro et al., 2010), a potential target of MD29 identified in our study. The active role of HSPs has been documented during different stages of the virus replication cycle and has been reported to cater broad functions during host infection (Santoro et al., 2010). They function as molecular chaperones and are constitutively expressed under a normal cellular environment. A study in Bombyx mori indicated that besides having a role in ubiquitin-proteasome system (UPS) HSC70-4 in the host, they are a novel component protein of budded virus (BV) and occlusion-derived virus (ODV) that helps them to propagate infection (Iwanaga et al., 2014). V-ATPase gene has been demonstrated to function in defense response against a nucleopolyhedrovirus in Bombyx mori (Lü et al., 2013). A study in Peregrinus maidis revealed that RNAi-mediated gene silencing of the V-ATPase gene caused higher mortality and lower fecundity (Yao et al., 2013). V-ATPase is one of the predicted targets of OrNV-MD507 and could likely suppress the defense system of *O. rhinoceros* for invasion. V-type proton ATPase subunit C, a component of V-ATPase protein complexes, is the crucial target for viral entry. It was demonstrated that the interruption of this protein leads to the inhibition of viral infection (Kohio and Adamson, 2013). Interestingly, studies also revealed that overexpression of specific subunits, including subunit C (target of MD1200) of V-ATPase protein complex, enhances viral infection (Adamson et al., 2011).

Another significantly upregulated miRNA in OrNV infected larvae is MD1200, which has predicted targets like tubulin polymerization-promoting protein homolog (TPPP), Ras-like protein 2, and Proline dehydrogenase 1. According to the study, TPPP plays crucial functions in myelinating oligodendrocytes, the most active cells in the central nervous (Lehotzky et al., 2010). Studies also revealed that overexpression of miRNAs targeting the TPPP inhibits cellular differentiation during development (Oláh et al., 2017). In OrNV infected larvae, this could arrest metamorphosis and succumb them to death. Ras-like proteins are among common

proteins that mediate viral infection to the host (Zhu et al., 2021). Proline dehydrogenase has a role in inducing intrinsic apoptosis, an important antiviral host defense mechanism (Ryerson et al., 2017; Zaghloul et al., 2017). Thus, MD1200 targeting Proline dehydrogenase could prevent early cell death that is triggered by OrNV infection. The majority of the predicted targets of MD29 are genes encoding proteins that have a WD40-repeat-containing domain. This category of proteins is known to play essential roles in numerous eukaryote-specific cellular processes such as G-protein-mediated signal transduction, transcription regulation, ubiquitin-dependent protein degradation, and chromatin modification (Hu et al., 2017).

Various other targets are also reported to have roles in mediating host-pathogen interaction. The potential targets like histone 4 and H3-K79-HMTase have been suggested to play a vital role in parasitism (Kumar et al., 2017). Studies associate these targets with the lysogenic quiescence or lysogenic phase of the virus life cycle (Wu et al., 2018). Sodium/potassium-transporting ATPase subunit alpha was found to be one of the potential targets of MD949. A previous study in the Chikunguinea virus revealed that functional sodium-potassium ATPase is required to mediate infection in the host body (Ashbrook et al., 2016). The ubiquitin-conjugating enzyme suppresses the innate sensing pathway in the host following infection (Davis and Gack, 2015; Heaton et al., 2015). TATA-binding protein (TBP), a potential target of MD31, aids in gene transcription and viral assembly (Quadt et al., 2006; Sampath and DeLuca, 2008). Cytochrome P450, which functions to metabolize a wide range of xenobiotic substances, was found as one of the major targets of MR1072, which are targeted by the pathogen during infection (Stavropoulou et al., 2018). Cytochrome P4510 might play a role in subverting the host resistance machinery, and multiple unique cytochrome P450 genes are found in giant viruses (Lamb et al., 2019).

One of the conserved OrNV miRNAs, i.e., MD783, targeted Mannan-binding lectin serine protease 2 involved in immune responses. The protein belongs to Mannose-binding lectin, a

pattern recognition system that attaches a tag of carbohydrate groups on the surface of microbial pathogens and activates the lectin. One study reveals that the mice mutant to this gene are more vulnerable to viral infection than the wild ones. It is likely that OrNV MD783 degrades or downregulates the genes to take over the immune system of the CRB for its sustenance (Fuchs et al., 2011). One of the vital proteins processed during the mature virus particle assembly, i.e., Gag-Pro-Pol polyprotein, is one of the critical predicted targets of MR1353. Gag-Pro-Pol polyprotein forms the virus's spherical core that encapsulates the genomic RNA-nucleocapsid complex, binds strongly to viral nucleic acids, and promotes their aggregation (Pettit et al., 2005). Another OrNV miRNA target, i.e., Ankyrin repeat domain-containing protein 29, is more commonly reported in eukaryotes and constitutes the viral domains of life. Owing to their active participation in protein-protein interactions and astonishingly found in a large number (Siozios et al., 2013).

Vast gene regulatory network mediates the host-virus interactions. While calcium is an integral component reported for viral function, calmodulin, a ubiquitously expressed Ca2+ binding protein (Crawford et al., 2012; Lewis et al., 1986), could be targeted by the virus to maintain its existence in the host body. In our study, calmodulin is found to be a potential target of MD29, a highly expressed miRNA in OrNV larvae. Reports also suggest that calcium ions and calmodulin regulate the activity of eEF2 kinase, which in turn control the action of the *EF2* gene (Khan et al., 2010). In our study, *EF2*, which functions in gene translation (Kaul et al., 2011), is predicted targets of miRNAs MD1025 and MR1353. We observed significant downregulation of *EF2* ($p < 0.005$) in OrNV treated larvae in our study, and this is in accordance with the established action of miRNA on target genes (Cannell et al., 2008). Transcriptional elongation mediated by *EF2* is a crucial step in regulating gene expression in prokaryotic, eukaryotic, and viral systems (Collins et al., 1996). *EF2* plays a role in viral infection as it involves the production of viral proteins like non-capsid proteins (Svitkin and

Agol, 1983). In our study, significant downregulation of *EF2* in the OrNV-treated larvae compared to control indicate a possible modulation of *EF2* by OrNV to reduce viral load in early stages of infection and the evasion of defense responses of *O. rhinoceros*.

Data from this study indicate that OrNV, through the deployment of miRNAs, has mechanisms in place for regulating its own genes as well as the genes of the host, the rhinoceros beetle. Viral miRNA effectors may be utilized for fine-tuning the temporal expressions of its genes during the viral life cycle in the host, in addition to targeting the host replication machinery and suppressing its defense systems. In a few other viruses- insect interactions, the role of specific miRNAs has been subjected to detailed analysis. As a matter of fact, the inventory of miRNAs identified in OrNV and their predicted targets reported here reconcile with the previous studies demonstrating the role of specific miRNA in facilitating insect-pathogen interactions. A study on these miRNAs and the target genes in OrNV resistant haplotypes may uncover the reasons for the loss of OrNV efficacy as a biocontrol agent. Identified miRNA repertoire of OrNV and their targets could be helpful for the development of pest control agents as well as in the design of management strategies.

5. Conclusion

To conclude, this study identified twenty-one miRNAs in the OrNV (Oryctes rhinoceros nudivirus) genome, of which seven are novel. Eight of the 21 miRNAs were validated using q-RT-PCR in OrNV infected larvae. The target of one miRNA was found to be downregulated in infected larvae compared to healthy larvae. Our findings form the premise for a better understanding of the regulation of *Oryctes* genes via miRNAs by OrNV. Most importantly, various miRNAs of OrNV and their targets could help devise efficient and environmentally friendly techniques for managing *O. rhinoceros*.

Declarations

Ethics approval and consent to participate (if applicable)

Not applicable.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that have, or could be perceived to have, influenced the work reported in this article.

Availability of data and materials

The transcriptome data used in this study have been deposited in the NCBI with accession numbers viz., SRX5004164, SRX5004165, SRX5004166, and SRX5004167.

Authors' contributions

T.G., G.A., and R.M.K. conceived and designed the experiments. J.A. facilitated insect rearing and sampling. K.A. carried out the experiments, analysed the data and wrote the manuscript. T.G., G.A., R.M.K., and J.A. reviewed and edited the manuscript. All authors approved this manuscript.

References

Adamson, A.L., Chohan, K., Swenson, J., LaJeunesse, D., 2011. A Drosophila Model for Genetic Analysis of Influenza Viral/Host Interactions. Genetics 189, 495–506. https://doi.org/10.1534/genetics.111.132290

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J Mol Biol 215, 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Arvind, K., Rajesh, M.K., Josephrajkumar, A., Grace, T., 2020. Dataset of de novo assembly and functional annotation of the transcriptome of certain developmental stages of coconut rhinoceros beetle, Oryctes rhinoceros L. Data in Brief 28, 105036. https://doi.org/10.1016/j.dib.2019.105036

Ashbrook, A.W., Lentscher, A.J., Zamora, P.F., Silva, L.A., May, N.A., Bauer, J.A., Morrison, T.E., Dermody, T.S., 2016. Antagonism of the Sodium-Potassium ATPase Impairs Chikungunya Virus Infection. mBio 7. https://doi.org/10.1128/mBio.00693-16

Bairoch, A., Apweiler, R., Wu, C.H., Barker, W.C., Boeckmann, B., Ferro, S., Gasteiger, E., Huang, H., Lopez, R., Magrane, M., Martin, M.J., Natale, D.A., O'Donovan, C., Redaschi, N., Yeh, L.-S.L., 2005. The Universal Protein Resource (UniProt). Nucleic Acids Res 33, D154–D159. https://doi.org/10.1093/nar/gki070

Bedford, G.O., 2018. Possibility of Evolution in Culture of the Oryctes Nudivirus of the Coconut Rhinoceros Beetle Oryctes rhinoceros (Coleoptera: Scarabaeidae: Dynastinae). Advances in Entomology 6, 27–33. https://doi.org/10.4236/ae.2018.61004

Bedford, G.O., 2013. Biology and Management of Palm Dynastid Beetles: Recent Advances. Annual Review of Entomology 58, 353–372. https://doi.org/10.1146/annurev-ento-120710-100547

Bruscella, P., Bottini, S., Baudesson, C., Pawlotsky, J.-M., Feray, C., Trabucchi, M., 2017. Viruses and miRNAs: More Friends than Foes. Frontiers in Microbiology 8, 824. https://doi.org/10.3389/fmicb.2017.00824

Cai, D., Yan, R., Xu, J.Z., Zhang, H., Shen, S., Mitra, B., Marchetti, A., Kim, E.S., Guo, H., 2020. Characterization of the Termini of Cytoplasmic Hepatitis B Virus Deproteinated Relaxed Circular DNA. J Virol 95, e00922-20. https://doi.org/10.1128/JVI.00922-20

Cannell, I.G., Kong, Y.W., Bushell, M., 2008. How do microRNAs Regulate Gene Expression. Biochemical Society Transactions 1224–1231.

Chaudhary, O.P., Mehra, R., Dalal, P.K., 2019. Recent trends in biointensive management of insect pests using microbes. pp. 317–341.

Collins, P.L., Hill, M.G., Cristina, J., Grosfeld, H., 1996. Transcription elongation factor of respiratory syncytial virus, a nonsegmented negative-strand RNA virus. Proc Natl Acad Sci U S A 93, 81–85.

Crawford, S.E., Hyser, J.M., Utama, B., Estes, M.K., 2012. Autophagy hijacked through viroporin-activated calcium/calmodulin-dependent kinase kinase-β signaling is required for rotavirus replication. Proc Natl Acad Sci U S A 109, E3405–E3413. https://doi.org/10.1073/pnas.1216539109

Davis, M.E., Gack, M.U., 2015. Ubiquitination in the antiviral immune response. Virology, 60th Anniversary Issue 479–480, 52–65. https://doi.org/10.1016/j.virol.2015.02.033

Dennis, G., Sherman, B.T., Hosack, D.A., Yang, J., Gao, W., Lane, H.C., Lempicki, R.A., 2003. DAVID: Database for Annotation, Visualization, and Integrated Discovery. Genome Biology 4, R60. https://doi.org/10.1186/gb-2003-4-9-r60

Enright, A.J., John, B., Gaul, U., Tuschl, T., Sander, C., Marks, D.S., 2003. MicroRNA targets in Drosophila. Genome Biol 5, R1. https://doi.org/10.1186/gb-2003-5-1-r1

Etebari, K., Parry, R., Beltran, M.J.B., Furlong, M.J., 2020. Transcription Profile and Genomic Variations of Oryctes Rhinoceros Nudivirus in Coconut Rhinoceros Beetles. J Virol 94. https://doi.org/10.1128/JVI.01097-20

Fuchs, A., Pinto, A.K., Schwaeble, W.J., Diamond, M.S., 2011. The lectin pathway of complement activation contributes to protection from West Nile virus infection. Virology 412, 101–109. https://doi.org/10.1016/j.virol.2011.01.003

Gkirtzou, K., Tsamardinos, I., Tsakalides, P., Poirazi, P., 2010. MatureBayes: A Probabilistic Algorithm for Identifying the Mature miRNA within Novel Precursors. PloS one 5, e11843. https://doi.org/10.1371/journal.pone.0011843

Grundhoff, A., Sullivan, C.S., 2011. Virus-encoded microRNAs. Virology 411, 325–343. https://doi.org/10.1016/j.virol.2011.01.002

Heaton, S.M., Borg, N.A., Dixit, V.M., 2015. Ubiquitin in the activation and attenuation of innate antiviral immunity. Journal of Experimental Medicine 213, 1–13. https://doi.org/10.1084/jem.20151531

Ho, C.K., Shuman, S., 2002. Bacteriophage T4 RNA ligase 2 (gp24.1) exemplifies a family of RNA ligases found in all phylogenetic domains. Proc Natl Acad Sci U S A 99, 12709–12714. https://doi.org/10.1073/pnas.192184699

Hu, X.-J., Li, T., Wang, Y., Xiong, Y., Wu, X.-H., Zhang, D.-L., Ye, Z.-Q., Wu, Y.-D., 2017. Prokaryotic and Highly-Repetitive WD40 Proteins: A Systematic Study. Sci Rep 7, 10585. https://doi.org/10.1038/s41598-017-11115-1

Huger, A.M., 2005. The Oryctes virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, Oryctes rhinoceros (Coleoptera: Scarabaeidae). Journal of Invertebrate Pathology, Special SIP Symposium Issue 89, 78–84. https://doi.org/10.1016/j.jip.2005.02.010

Iwanaga, M., Shibano, Y., Ohsawa, T., Fujita, T., Katsuma, S., Kawasaki, H., 2014. Involvement of HSC70-4 and other inducible HSPs in Bombyx mori nucleopolyhedrovirus infection. Virus Research 179, 113–118. https://doi.org/10.1016/j.virusres.2013.10.028

Jiang, P., Wu, H., Wang, W., Ma, W., Sun, X., Lu, Z., 2007. MiPred: classification of real and pseudo microRNA precursors using random forest prediction model with combined features. Nucleic Acids Res 35, W339–W344. https://doi.org/10.1093/nar/gkm368

Kaul, G., Pattan, G., Rafeequi, T., 2011. Eukaryotic elongation factor-2 (eEF2): its regulation and peptide chain elongation. Cell Biochemistry and Function 29, 227–234. https://doi.org/10.1002/cbf.1740

Khan, A.A., Dace, D.S., Ryazanov, A.G., Kelly, J., Apte, R.S., 2010. Resveratrol Regulates Pathologic Angiogenesis by a Eukaryotic Elongation Factor-2 Kinase-Regulated Pathway. The American Journal of Pathology 177, 481–492. https://doi.org/10.2353/ajpath.2010.090836

Kohio, H.P., Adamson, A.L., 2013. Glycolytic control of vacuolar-type ATPase activity: A mechanism to regulate influenza viral infection. Virology 444, 301–309. https://doi.org/10.1016/j.virol.2013.06.026

Kumar, S., Jung, J.-K., Kim, Y., 2017. Characterization of joining sites of a viral histone H4 on host insect chromosomes. PLOS ONE 12, e0177066. https://doi.org/10.1371/journal.pone.0177066

Lamb, D.C., Follmer, A.H., Goldstone, J.V., Nelson, D.R., Warrilow, A.G., Price, C.L., True, M.Y., Kelly, S.L., Poulos, T.L., Stegeman, J.J., 2019. On the occurrence of cytochrome P450 in viruses. Proc Natl Acad Sci U S A 116, 12343–12352. https://doi.org/10.1073/pnas.1901080116

Lee, S., Park, K.-H., Nam, S.-H., Kwak, K.-W., Choi, J.-Y., 2015. First Report of Oryctes rhinoceros nudivirus (Coleoptera: Scarabaeidae) Causing Severe Disease in Allomyrina dichotoma in Korea. Journal of Insect Science 15. https://doi.org/10.1093/jisesa/iev002

Lehotzky, A., Lau, P., Tőkési, N., Muja, N., Hudson, L.D., Ovádi, J., 2010. Tubulin polymerization-promoting protein (TPPP/p25) is critical for oligodendrocyte differentiation. Glia 58, 157–168. https://doi.org/10.1002/glia.20909

Lewis, M.G., Chang, J.Y., Olsen, R.G., Fertel, R.H., 1986. Identification of calmodulin activity in purified retroviruses. Biochemical and Biophysical Research Communications 141, 1077–1083. https://doi.org/10.1016/S0006-291X(86)80153-X

Lü, P., Xia, H., Gao, L., Pan, Y., Wang, Y., Cheng, X., Lü, H., Lin, F., Chen, L., Yao, Q., Liu, X., Tang, Q., Chen, K., 2013. V-ATPase Is Involved in Silkworm Defense Response against Bombyx mori Nucleopolyhedrovirus. PLOS ONE 8, e64962. https://doi.org/10.1371/journal.pone.0064962

Marshall, S.D.G., Moore, A., Vaqalo, M., Noble, A., Jackson, T.A., 2017. A new haplotype of the coconut rhinoceros beetle, Oryctes rhinoceros, has escaped biological control by Oryctes rhinoceros nudivirus and is invading Pacific Islands. Journal of Invertebrate Pathology 149, 127–134. https://doi.org/10.1016/j.jip.2017.07.006

Miller, A.C., Voelker, L.H., Shah, A.N., Moens, C.B., 2015. Neurobeachin Is Required Postsynaptically for Electrical and Chemical Synapse Formation. Current Biology 25, 16–28. https://doi.org/10.1016/j.cub.2014.10.071

Mishra, R., Kumar, A., Ingle, H., Kumar, H., 2020. The Interplay Between Viral-Derived miRNAs and Host Immunity During Infection. Frontiers in Immunology 10, 3079. https://doi.org/10.3389/fimmu.2019.03079

Nebl, G., Mermod, N., Cato, A.C., 1994. Post-transcriptional down-regulation of expression of transcription factor NF1 by Ha-ras oncogene. Journal of Biological Chemistry 269, 7371–7378. https://doi.org/10.1016/S0021-9258(17)37294-0

O'Brien, J., Hayder, H., Zayed, Y., Peng, C., 2018. Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Frontiers in Endocrinology 9, 402. https://doi.org/10.3389/fendo.2018.00402

Oláh, J., Szénási, T., Szunyogh, S., Szabó, A., Lehotzky, A., Ovádi, J., 2017. Further evidence for microtubule-independent dimerization of TPPP/p25. Sci Rep 7, 40594. https://doi.org/10.1038/srep40594

Ospina-Bedoya, M., Campillo-Pedroza, N., Franco-Salazar, J.P., Gallego-Gómez, J.C., 2014. Computational Identification of Dengue Virus MicroRNA-Like Structures and Their Cellular Targets. Bioinform Biol Insights 8, BBI.S13649. https://doi.org/10.4137/BBI.S13649

Pettit, S.C., Lindquist, J.N., Kaplan, A.H., Swanstrom, R., 2005. Processing sites in the human immunodeficiency virus type 1 (HIV-1) Gag-Pro-Pol precursor are cleaved by the viral protease at different rates. Retrovirology 2, 66. https://doi.org/10.1186/1742-4690-2-66

Quadt, I., Günther, A.K., Voß, D., Schelhaas, M., Knebel-Mörsdorf, D., 2006. TATA-binding protein and TBP-associated factors during herpes simplex virus type 1 infection: Localization at viral DNA replication sites. Virus Research 115, 207–213. https://doi.org/10.1016/j.virusres.2005.09.010

Ryerson, M.R., Richards, M.M., Kvansakul, M., Hawkins, C.J., Shisler, J.L., n.d. Vaccinia Virus Encodes a Novel Inhibitor of Apoptosis That Associates with the Apoptosome. Journal of Virology 91, e01385-17. https://doi.org/10.1128/JVI.01385-17

Sampath, P., DeLuca, N.A., 2008. Binding of ICP4, TATA-Binding Protein, and RNA Polymerase II to Herpes Simplex Virus Type 1 Immediate-Early, Early, and Late Promoters in Virus-Infected Cells. J Virol 82, 2339–2349. https://doi.org/10.1128/JVI.02459-07

Santoro, M.G., Amici, C., Rossi, A., 2010. Role of Heat Shock Proteins in Viral Infection, in: Pockley, A.G., Calderwood, S.K., Santoro, M.G. (Eds.), Prokaryotic and Eukaryotic Heat Shock Proteins in Infectious Disease, Heat Shock Proteins. Springer Netherlands, Dordrecht, pp. 51–84. https://doi.org/10.1007/978-90-481-2976-8_3

Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Res 13, 2498–2504. https://doi.org/10.1101/gr.1239303

Singh, C.P., Singh, J., Nagaraju, J., 2014. bmnpv-miR-3 facilitates BmNPV infection by modulating the expression of viral P6.9 and other late genes in Bombyx mori. Insect Biochemistry and Molecular Biology 49, 59–69. https://doi.org/10.1016/j.ibmb.2014.03.008

Siozios, S., Ioannidis, P., Klasson, L., Andersson, S.G.E., Braig, H.R., Bourtzis, K., 2013. The Diversity and Evolution of Wolbachia Ankyrin Repeat Domain Genes. PLOS ONE 8, e55390. https://doi.org/10.1371/journal.pone.0055390

Skalsky, R.L., Cullen, B.R., 2010. Viruses, microRNAs, and Host Interactions. Annual Review of Microbiology 64, 123–141. https://doi.org/10.1146/annurev.micro.112408.134243

Stavropoulou, E., Pircalabioru, G.G., Bezirtzoglou, E., 2018. The Role of Cytochromes P450 in Infection. Frontiers in Immunology 9, 89. https://doi.org/10.3389/fimmu.2018.00089

Svitkin, Y.V., Agol, V.I., 1983. Translational Barrier in Central Region of Encephalomyocarditis Virus Genome. European Journal of Biochemistry 133, 145–154. https://doi.org/10.1111/j.1432-1033.1983.tb07440.x

Tanaka, N., Shuman, S., 2011. RtcB Is the RNA Ligase Component of an Escherichia coli RNA Repair Operon*. Journal of Biological Chemistry 286, 7727–7731. https://doi.org/10.1074/jbc.C111.219022

Teng, Y., Wang, Y., Zhang, X., Liu, W., Fan, H., Yao, H., Lin, B., Zhu, P., Yuan, W., Tong, Y., Cao, W., 2015. Systematic Genome-wide Screening and Prediction of microRNAs in EBOV During the 2014 Ebolavirus Outbreak. Sci Rep 5, 9912. https://doi.org/10.1038/srep09912

Trotman, L.C., Mosberger, N., Fornerod, M., Stidwill, R.P., Greber, U.F., 2001. Import of adenovirus DNA involves the nuclear pore complex receptor CAN/Nup214 and histone H1. Nat Cell Biol 3, 1092–1100. https://doi.org/10.1038/ncb1201-1092

Wang, W., Vinocur, B., Shoseyov, O., Altman, A., 2004. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends in Plant Science 9, 244–252. https://doi.org/10.1016/j.tplants.2004.03.006

Wang, Z., Ye, X., Zhou, Y., Wu, X., Hu, R., Zhu, J., Chen, T., Huguet, E., Shi, M., Drezen, J.-M., Huang, J., Chen, X., 2021. Bracoviruses recruit host integrases for their integration into caterpillar's genome. PLOS Genetics 17, e1009751. https://doi.org/10.1371/journal.pgen.1009751

Wang, Zhi-zhi, Ye, X., Shi, M., Li, F., Wang, Ze-hua, Zhou, Y., Gu, Q., Wu, X., Yin, C., Guo, D., Hu, R., Hu, N., Chen, T., Zheng, B., Zou, J., Zhan, L., Wei, S., Wang, Y., Huang, J., Fang, X., Strand, M.R., Chen, X., 2018. Parasitic insect-derived miRNAs modulate host development. Nat Commun 9, 2205. https://doi.org/10.1038/s41467-018-04504-1

Wu, P.-C., Lin, Y.-H., Wu, T.-C., Lee, S.-T., Wu, C.-P., Chang, Y., Wu, Y.-L., 2018. MicroRNAs derived from the insect virus HzNV-1 promote lytic infection by suppressing histone methylation. Sci Rep 8, 17817. https://doi.org/10.1038/s41598-018-35782-w

Yao, J., Rotenberg, D., Afsharifar, A., Barandoc-Alviar, K., Whitfield, A.E., 2013. Development of RNAi Methods for Peregrinus maidis, the Corn Planthopper. PLOS ONE 8, e70243. https://doi.org/10.1371/journal.pone.0070243

Zaghloul, H.A.H., Hice, R., Arensburger, P., Federici, B.A., n.d. Transcriptome Analysis of the Spodoptera frugiperda Ascovirus In Vivo Provides Insights into How Its Apoptosis Inhibitors and Caspase Promote Increased Synthesis of Viral Vesicles and Virion Progeny. Journal of Virology 91, e00874-17. https://doi.org/10.1128/JVI.00874-17

Zhu, J., Eid, F.E., Tong, L., Zhao, W., Wang, W., Heath, L.S., Kang, L., Cui, F., 2021. Characterization of protein–protein interactions between rice viruses and vector insects. Insect Science 28, 976–986. https://doi.org/10.1111/1744-7917.12840