Groundnut Bud Necrosis Virus Modulates the Expression of Innate Immune, Endocytosis, and Cuticle Development-Associated Genes to Circulate and Propagate in Its Vector, *Thrips palmi*

Deepak Kumar Mahanta¹,², Sumit Jangra¹, Priti⁴, Amalendu Ghosh⁴*, Parva Kumar Sharma³, Mir Asif Iquebal³, Sarika Jaiswal³, Virendra Kumar Baranwal¹, Vinay Kumari Kalia² and Subhas Chander⁴

Thrips palmi (Thysanoptera: Thripidae) is the predominant tospovirus vector in Asia-Pacific region. It transmits economically damaging groundnut bud necrosis virus (GBNV, family Tospoviridae) in a persistent propagative manner. Thrips serve as the alternate host, and virus reservoirs making tospovirus management very challenging. Insecticides and host plant resistance remain ineffective in managing thrips–tospoviruses. Recent genomic approaches have led to understanding the molecular interactions of thrips–tospoviruses and identifying novel genetic targets. However, most of the studies are limited to *Frankliniella* species and tomato spotted wilt virus (TSWV). Amidst the limited information available on *T. palmi*–tospovirus relationships, the present study is the first report of the transcriptome-wide response of *T. palmi* associated with GBNV infection. The differential expression analyses of the triplicate transcriptome of viruliferous vs. nonviruliferous adult *T. palmi* identified a total of 2,363 (1,383 upregulated and 980 downregulated) significant transcripts. The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses showed the abundance of differentially expressed genes (DEGs) involved in innate immune response, endocytosis, cuticle development, and receptor binding and signaling that mediate the virus invasion and multiplication in the vector system. Also, the gene regulatory network (GRN) of most significant DEGs showed the genes like ABC transporter, cytochrome P450, endocuticle structural glycoprotein, gamma-aminobutyric acid (GABA) receptor, heat shock protein 70, larval and pupal cuticle proteins, nephrin, proline-rich protein, sperm-associated antigen, UHRF1-binding protein, serpin, tyrosine–protein kinase receptor, etc., were enriched with higher degrees of interactions. Further, the expression of the candidate genes in response to GBNV infection was validated in reverse transcriptase-quantitative real-time PCR (RT-qPCR). This study
INTRODUCTION

Thrips are minute, fringed winged insects that belong to the family Thripidae in the order Thysanoptera. Globally, thrips are considered as one of the most economically damaging pests of a wide range of food, feed, and fiber crops (Riley et al., 2011). Thrips rasp the soft plant tissues and suck sap from leaves, stems, flowers, and fruits. The infested plant parts develop distinctive silvery or bronze scarring. High incidence leads to curling, rolling, folding, distortion of leaves, and deformation of fruits. Besides, thrips are sole transmitters of tospoviruses (genus *Orthotospovirus*, family *Tospoviridae*, and order *Bunyavirales*) that cause significant economic losses to crop production across the globe (Ullman et al., 1992, 1997; Whitfield et al., 2005; Oliver and Whitfield, 2016). Annual losses due to tospovirus outbreaks are estimated to cost growers over US$ 1 billion worldwide (Pappu et al., 2009). To date, 16 thrips species are reported to vector 29 tospoviruses (Rotenberg et al., 2015; Ghosh et al., 2017). Tospoviruses propagate within thrips and thrips serve as effective alternate hosts and virus reservoirs which make tospovirus management very difficult (Ullman et al., 2002; Whitfield et al., 2005).

Melon thrips (*Thrips palmi* Karny) is a predominant thrips species in Asia and has spread to the Pacific, Florida, Hawaii, the Caribbean, South America, West Africa, and Australia (Bhatti, 1980; Johnson, 1986; Pantono et al., 1988; Cooper, 1991; Palmer, 1992; Monteiro et al., 1995; Guyot, 1998; Kawai, 2001; Cannon et al., 2007; Ghosh et al., 2020). It infects over 200 plant species of family *Asteraceae*, *Cucurbitaceae*, *Fabaceae*, and *Solanaceae* (Walker, 1992; Cermeli and Montagne, 1993). *T. palmi* is known to transmit seven species of tospoviruses, such as calla lily chlorotic spot virus (CCCSV), capsicum chlorosis virus (CaCV), groundnut bud necrosis virus (GBNV), melon yellow spot virus (MYSV), tomato necrotic ringspot virus (TNRV), watermelon bud necrosis virus (WBNV), and watermelon silver mottle virus (WSMoV; Ghosh et al., 2017, 2020). GBNV is the predominant tospovirus in Asia causing annual losses of approximately US$ 89 million. As much as 70%–90% of losses in groundnut were recorded due to infection of GBNV in India (Singh and Srivastava, 1995). Outbreaks of GBNV in tomatoes caused up to 100% disease incidence from 2003 to 2006 in India (Kunkalikar et al., 2011). Yield losses up to 29% have been reported in potatoes due to GBNV infection (Singh et al., 1997). Several management strategies have been adopted around the world, with insecticides and host plant resistance being the core components for thrips and tospovirus management, respectively. Farmers worldwide spend more than US$ 50 million annually to manage this vector. Insecticides are largely ineffective due to the emergence of resistant thrips population. Genetic host plant resistance is not available for all the crop species that are infected by thrips–tospoviruses. Understanding the interactions between thrips and tospovirus will be helpful to identify novel genetic targets to interrupt the interrelationship and restrict the epidemics of thrips–tospoviruses.

Various genomic approaches have been reported to understand the molecular interactions of thrips and tospoviruses. However, most of these studies are focused on tomato spotted wilt virus (*TSVW*) and *Frankliniella* species (Badillo-Vargas et al., 2012; Zhang et al., 2013; Stafford-Banks et al., 2014; Schneweis et al., 2017; Han and Rotenberg, 2021). *Frankliniella occidentalis* genes associated with host defense, insect cuticle structure and development, metabolism, and transport are regulated by TSVW infection (Schneweis et al., 2017). Genes associated with intracellular transport, development, and immune responses are regulated by TSVW infection in *Frankliniella fusca* (Shrestha et al., 2017). Transcriptome-wide responses of *T. palmi* associated with CaCV infection revealed upregulation of innate immune, digestion of proteins, and lipids-related genes, while the genes associated with the structural constituent of the cuticle were downregulated (Gamage et al., 2018). Tospovirus (WBNV) infects the epidermal cells of the anterior midgut of *T. palmi* and spreads to primary salivary glands via connecting ligaments and other parts of the alimentary canal (Ghosh et al., 2021). Tospovirus (WSMoV) propagates in the primary salivary glands and midgut of *T. palmi* (Mou et al., 2021). GBNV infection was found to negatively alter the survivability, adult longevity, and reproductive potential of *T. palmi* (Ghosh et al., 2019). Host plant also influences the biology and transmission of tospovirus (WBNV) by *T. palmi* (Dhall et al., 2021). Little is known about the differentially expressed genes (DEGs), pathways, and molecular interactions of *T. palmi* in response to GBNV infection. The present study reports the candidate genes of *T. palmi* responsive to GBNV infection through a comparative transcriptomic approach that would be novel targets for generic management of the thrips–tospovirus complex.

MATERIALS AND METHODS

Establishing an Isofemale Population of *Thrips palmi*

An isofemale population of *T. palmi* maintained at Advanced Centre for Plant Virology, Indian Agricultural Research Institute (IARI), New Delhi, was used in the study. The identification of *T. palmi* was based on the morphological keys and mitochondrial cytochrome oxidase subunit I (mtCOI) sequences amplified in PCR using primer pairs, LCO 1490 and HCO 2198 (Folmer et al., 1994; Jangra et al., 2020). The population was established from a single adult female of *T. palmi* on eggplant (var. Navkiran, Mahyco). The population has been

**Keywords:** melon thrips, tospovirus, virus–vector relationship, RT-qPCR, transcriptome
maintained at the thrips rearing facility under controlled environmental conditions at 28 ± 1°C temperature, 60 ± 10% relative humidity, and 16 h of light–8 h of the dark since 2018. Fresh virus-free healthy eggplants were supplied as and when required. The isofemale population has been used in replicate throughout the experiment.

Establishment of Pure Culture of GBNV
The initial inoculum of GBNV was collected from the pure culture maintained at Advanced Centre for Plant Virology, IARI. Healthy cowpea plants (var. Pusa Komal) were raised from seeds in a plant growth chamber (A2000, Conviron, Canada). The healthy cowpea plants at a two-leaf stage were sap-inoculated with GBNV as described by Ghosh et al. (2021). Briefly, the sap was extracted by grinding the inoculum in a pre-sterilized mortar on ice. Ice-cold sodium phosphate buffer (0.01 M, pH 7.0) was added at tissue to buffer ratio of 1:6 wt/vol. β-mercaptoethanol (0.2%) was added while grinding the tissue. A pinch of Celite was dusted on the cowpea plants. The sap was applied to the leaves by gently rubbing with gloved hands. The inoculated plants were washed with sterile distilled water after a while. The inoculated plants were maintained at 25 ± 1°C, 60 ± 10% relative humidity, and 16 h of light–8 h of dark under insect-proof conditions. Nutrient solution containing macro, meso, and trace elements at appropriate concentrations was provided to plants and regularly monitored for symptom appearance. All the inoculated plants were tested for symptom appearance. The inoculated plants were transplanted into a T100 thermocycler (Bio-Rad, United States) by primer annealing at 25°C for 10 min, reverse transcription at 50°C for 30 min and followed by enzyme inactivation at 85°C for 5 min. PCR was carried out in 25 μl reactions containing 2 μl cDNA, 2.5 μl 10X PCR buffer (Thermo Fisher Scientific), 0.4 μM each of forward and reverse primers, 260 μM dNTP mix (Thermo Fisher Scientific), and 2 U DreamTaq polymerase (Thermo Fisher Scientific). PCR was performed in a T100 Thermal Cycler with 95°C for 5 min followed by 30 cycles of 94°C for 30 s, 59°C for 45 s, and 72°C for 1 min followed by a final extension at 72°C for 7 min. RT-PCR products were resolved on 1% agarose gel stained with GoodView (BR Biochem, India) and observed in a gel documentation system (MasteroGen Inc., Taiwan). The purified product was sequenced bidirectional. The sequences were processed by BioEdit and BLASTn was performed to check the species homology. The consensus sequence was submitted to GenBank.

Diagnosis of GBNV Infection by RT-PCR
The total RNA was isolated from cowpea plants using RNeasy Plant Mini Kit (Qiagen, Germantown, MD, United States). Complementary DNA (cDNA) was synthesized with random primers using FIREScript RT cDNA synthesis kit (Solis Biodyne, Estonia). The reaction mixture contained 1X RT reaction buffer, 1.0 μg template RNA, 5 μM random primers, 2 mM dNTP mix, 10-unit FIREScript RT, and 20-unit RiboGrip RNase inhibitor. The reverse transcription was carried out in a T100 thermocycler (Bio-Rad, United States) by primer annealing at 25°C for 10 min, reverse transcription at 50°C for 30 min and followed by enzyme inactivation at 85°C for 5 min. PCR was carried out in 25 μl reactions containing 2 μl cDNA, 2.5 μl 10X PCR buffer (Thermo Fisher Scientific), 0.4 μM each of forward and reverse primers, 260 μM dNTP mix (Thermo Fisher Scientific), and 2 U DreamTaq polymerase (Thermo Fisher Scientific). PCR was performed in a T100 Thermal Cycler with 95°C for 5 min followed by 30 cycles of 94°C for 30 s, 59°C for 45 s, and 72°C for 1 min followed by a final extension at 72°C for 7 min. RT-PCR products were resolved on 1% agarose gel stained with GoodView (BR Biochem, India) and observed in a gel documentation system (MasteroGen Inc., Taiwan). The purified product was sequenced bidirectional. The sequences were processed by BioEdit and BLASTn was performed to check the species homology. The consensus sequence was submitted to GenBank.

Generation of Viruliferous and Nonviruliferous Thrips palmi Population
The freshly emerged first instar larvae (L1, less than 24 h old) of T. palmi were collected using a Camel hairbrush from the isofemale population on eggplants and used for GBNV acquisition in three replicates to generate the viruliferous population. The virus acquisition setup was established as described by Ghosh et al. (2021). About 50 T. palmi L1s in each replicate were placed on a GBNV-infected leaf and allowed to feed for 24 h at 28 ± 1°C and 60 ± 10% relative humidity. The setup was continuously monitored to confirm the feeding of larvae on the leaf. After the acquisition access period, the larvae were transferred on a detached healthy cowpea leaf. The petiole of the detached leaf was inserted in a slant of 0.8% dextrose agar within an insect breeding dish (10 cm diameter, 4 cm height, SPL Life Sciences, Korea). The GBNV-exposed T. palmi were reared up to the adult stage on cowpea leaves at 28 ± 1°C, 60 ± 10% relative humidity, and 16 h of light–8 h of dark. The breeding dishes were regularly monitored and supplied with fresh leaves as and when required. To generate nonviruliferous T. palmi adults, the L1s were released on the healthy cowpea leaves placed on the agar slant within the insect breeding dishes in three replicates as described above. Adults were collected immediately after their emergence and used in the study. Three sets of viruliferous (designated as TpTrI1, TpTrI2, and TpTrI3) and nonviruliferous (TpTrH1, TpTrH2, and TpTrH3) T. palmi adult populations were developed. Each set was divided into two parts. One part was used for transcriptome sequencing and another part was preserved at −80°C for gene expression analysis in reverse transcriptase-quantitative real-time PCR (RT-qPCR).

Infection of GBNV in T. palmi was confirmed by randomly collecting five individual adults from each of the viruliferous and nonviruliferous insect populations and testing in RT-PCR. Total RNA from adults of T. palmi was isolated using NucleoSpin RNA XS (Macherey-Nagel, Germany), and RT-PCR was performed as described above.

RNA Extraction, Library Preparation, and Sequencing
Three sets of viruliferous (TpTrI1, TpTrI2, and TpTrI3) and nonviruliferous (TpTrH1, TpTrH2, and TpTrH3) adults of T. palmi were subjected to total RNA isolation using TRIzol (Invitrogen, United States). Each set comprised 10 adult individuals that included both females and males (1:1). RNA quality was checked using RNA 6000 Nano Kit (Agilent Technologies, United States) on 2100 Bioanalyzer (Agilent Technologies) with a minimum RNA Integrity Number (RIN) value of 7. RNA concentrations were determined with a NanoDrop ND-8000 spectrophotometer (Thermo Fisher Scientific). RNA-Seq libraries for all samples were prepared using NEBNext UltraII RNA library preparation kit for Illumina (New England Biolabs, United States), and sequencing was done in a single HiSEQ 4,000 (Illumina, Inc. United States) lane using 150 bp paired-end chemistry. The library preparation and sequencing were done by commercial service providers.
Mahanta et al. Transcriptome of Thrips palmi

Briefly, total RNA was used to purify poly(A) messenger RNA (mRNA) using oligo-dT beads. Magnetic beads were used for two rounds of purification. During the second elution of the poly-A RNA, the RNA was also fragmented into 200–500 bp pieces in the presence of divalent cations at 94°C for 5 min using an ultrasonicator. The cleaved RNA fragments were copied into first-strand cDNA using SuperScript-II Reverse Transcriptase (Thermo Fischer Scientific) and random primers. After second-strand cDNA synthesis, fragments were end-repaired and A-tailed, and indexed adapters were ligated. The products were purified and enriched with PCR to create the final cDNA library. The tagged cDNA libraries were pooled in equal ratios and used for 2×150 bp paired-end sequencing on a single lane of the Illumina HiSeq4000. Illumina clusters were generated and were loaded onto Illumina Flow Cell on Illumina HiSeq 4000 instrument and sequencing was carried out. After sequencing, the samples were demultiplexed and the indexed adapter sequences were trimmed using the CASAVA v1.8.2 software (Illumina, Inc.).

Data Preprocessing, Assembly, and Differential Gene Expression

The obtained low-quality raw reads were preprocessed to remove the adaptor contamination using Trim Galore v0.4.1 with the quality score <20%, and trimming ambiguous “N” nucleotides with a ratio of “N”>5%. A reference genome index was established using BWA v0.7.5 (Li and Durbin, 2009). The processed clean paired-end reads were used to map the reference genome of T. palmi (Guo et al., 2020) by BWA-MEM (Li, 2013). Read numbers mapped to every gene were counted using Samtools v0.1.19 software. Differential expression between the viruliferous and nonviruliferous T. palmi populations was analyzed using the DESeq R package. An absolute value of log fold change >2 and value of p < 0.05 were used as the thresholds to judge the significance of gene expression differences.

GO and KEGG Pathway Enrichment of Differentially Expressed Genes

Gene annotations and functional enrichment analysis including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) biological pathways were performed to identify the DEGs that were significantly enriched in GO terms or biological pathways post-GBNV infection. GO enrichment analysis for the identified GO terms was performed using the Fisher’s exact test available in the Blast2Go program (Conesa and Götz, 2008). Gene annotations against the Uniprot GO database were performed by aligning DEGs to the NR database using Blast2Go v5.2.5 software. GO terms were also assigned using the QuickGO online search tool. KEGG pathway enrichment analysis of DEGs was performed using the KEGG database resource to identify the pathways that were differentially regulated between viruliferous and nonviruliferous T. palmi with corrected value of p < 0.05. KEGG pathway enrichment was performed by calculating the enrichment factor for all the identified KEGG pathways. The top 20 highly enriched pathways were plotted based on the rich factor.

Gene Regulatory Network Analysis

The top 20 genes each from up- and downregulated DEGs were selected for the construction of gene regulatory networks (GRNs). These DEGs were filtered based on the log fold change values. The network was analyzed and visualized using Cytoscape v3.7.2, where gene correlation was computed using the Pearson’s correlation coefficient of the normalized expression values (Shannon et al., 2003). Network Analyzer plug-in was used to estimate the network centrality and topology.

Validation of RNA-Seq Expression With Real-Time RT-qPCR

To validate the differential gene expression data by RNA-Seq analysis, a few highly up- and downregulated DEGs of T. palmi were selected to assess the gene expression in RT-qPCR. A part of the preserved viruliferous and nonviruliferous T. palmi populations was used in RT-qPCR analysis. Initially, three sets of primers were designed for each of the target genes, and finally, one pair of primers for each gene was selected after validation. β-tubulin was considered as an endogenous control. All the primer sets were validated and optimized in a gradient PCR. Following thermal cycling conditions were followed for validation and optimization of target genes and endogenous control primers: initial denaturation at 95°C for 5 min, then 35 cycles of 95°C for 40 s, 50°C–60°C (depending upon the Tm of primer pairs) for 40 s, and 72°C for 40 s followed by a final extension at 72°C for 10 min. The primer pairs that produced a single specific sharp band at the same PCR conditions for β-tubulin primers were further selected for RT-qPCR experiment. The list of primers that were validated and used in RT-qPCR assay is included in Supplementary Table 1.

The relative expressions of target genes were estimated by RT-qPCR following 2^–△△CT method (Livak and Schmittgen, 2001). Firstly, total RNA was isolated from viruliferous (TpTrH1, TpTrH2, and TpTrH3) and nonviruliferous (TpTrH1, TpTrH2, and TpTrH3) thrips samples stored at −80°C using NucleoSpin RNA XS. cDNA was synthesized with oligo (dT) primer using FIREScript RT cDNA synthesis kit. The reaction mixture contained 1X RT reaction buffer, 1.0 µg template RNA, 5 µM oligo dT primer, 2 mM dNTP mix, 10-unit FIREScript RT, and 20-unit RiboGrip RNase inhibitor. The reverse transcription was carried out in a T100 thermocycler at 50°C for 30 min followed by enzyme inactivation at 85°C for 5 min. RT-qPCR was performed in an Insta Q 48M (Himedia, India) using the DyNAmo ColorFlash SYBR Green qPCR Kit (Thermo Fisher Scientific). A 20µl of RT-qPCR reaction mixture consisted of 10µl of 1X DyNAmo ColorFlash SYBR Green Master Mix, 0.25µM ROX passive reference dye, 0.2 µM each forward and reverse primer, and 2 µl template cDNA. Thermal cycling was performed as initial denaturation at 95°C for 5 min, 35 cycles

1 https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/
2 http://www.bioconductor.org/packages/release/bioc/html/DESeq.html
3 http://www.geneontology.org/
4 https://www.ebi.ac.uk
5 http://www.genome.jp/kegg

Frontiers in Microbiology | www.frontiersin.org 4 March 2022 | Volume 13 | Article 773238
of 95°C for 40 s, annealing at 53–59°C (depending upon the 
Tm of primer pairs) for 40 s, and 72°C for 40 s. Since SYBR 
Green I dye binds nonspecifically to any double-stranded DNA, 
a dissociation or melting stage was added after every reaction 
to determine the specificity of the amplicons based on the 
melting curves. The RT-qPCR was performed with three biological 
and two technical replicates. Log₂ fold change value was calculated and relative expression of mRNA was determined by normalized log₂ \( \Delta \Delta CT \) (Livak and Schmittgen, 2001) values of viruliferous 
(TpTrI) in comparison to nonviruliferous (TpTrH) \( \Delta \Delta CT \). 
\( \Delta \Delta CT \) was calculated as average \( \Delta C_T \) of nonviruliferous replicates—\( \Delta C_T \) of each replicate. The average 
log₂ \( \Delta \Delta CT \) was calculated for both the viruliferous and 
nonviruliferous populations and transformed to log₂ \( \Delta \Delta CT \). 
The log₂-fold change of the target gene expression in viruliferous 
\( T. \) palmi population was estimated by normalizing the fold 
change values of the nonviruliferous population. The relative 
expressions of target genes post-GBNV exposure in RT-qPCR 
assay were compared with log₂-fold changes obtained through 
RNA-Seq analysis, and Pearson’s correlation coefficient was 
calculated using CORREL function in MS Excel.

RESULTS

\( \textbf{T. palmi} \) Population

The isofemale \( T. \) palmi population generated from a single 
adult female was confirmed on the basis of morphological 
characters. The adults of \( T. \) palmi were yellow in color measuring 
about 0.8 mm–1 mm in length. Three brick red ocelli were 
visible in a triangular fashion between the compound eyes. A 
pair of interocellar setae originated outside the ocellar triangle. 
The head was quadrangular in shape with seven segmented 
antennae. Female adults were comparatively larger than males. 
Female adults had sharp ovipositor at the apex of the abdomen 
where males were with rounded apex. Further, the nucleotide 
sequence of mtCOI confirmed the identity as \( T. \) palmi. A 
657 bp fragment of mtCOI was amplified using primer pairs 
LCO 1490 and HCO 2198 (Folmer et al., 1994). The nucleotide 
sequence of the amplified product showed 100% homology to 
other \( T. \) palmi isolates (MN594549, NC_039437, and KX622379) 
available in GenBank. The sequence can be retrieved from the 
GenBank with Accession number MW020349.

GBNV Infection of \( T. \) palmi and Cowpea 
Plant

The initial inoculum of GBNV was collected from the pure 
culture (tomato isolate) maintained at Advanced Centre for 
Plant Virology, IARI. Healthy cowpea plants (\( \text{Vigna unguiculata} \) 
var. Pusa Komal) were sap-inoculated with GBNV. Characteristic 
symptoms of GBNV were recorded on sap-inoculated cowpea 
plants 10–14 days post-inoculation (dpi). Local lesions appeared 
on inoculated leaves and yellow necrotic spots with multiple 
rings-like structures were visible on systemic leaves. RT-PCR 
with GBNV-specific primers produced an amplicon of 1.8 kb 
for all symptomatic samples that confirmed the GBNV infection 
in inoculated plants. No amplification was observed in RT-PCR 
for healthy plant samples. Further, the nucleotide sequence of 
the amplified product showed more than 97% identity to GBNV. 
The sequence can be retrieved from the GenBank with Accession 
No. MN566913.

The isofemale \( T. \) palmi adults in three replicates (TpTrH1, 
TpTrH2, and TpTrH3) exposed to GBNV-infected cowpea plants 
during the L1 stage were tested in RT-PCR. A 1.8 kb product 
was visualized on agarose gel that confirmed the infection of 
GBNV in \( T. \) palmi adults. \( T. \) palmi populations (TpTrH1, TpTrH2, 
and TpTrH3) that were exposed to healthy cowpea plants did 
not produce any GBNV-specific amplification in RT-PCR.

Data Preprocessing, Assembly, and 
Differential Expression Analysis

\( T. \) palmi transcriptome data generated a total of 156,127,441 
raw reads of 150bp from three replicated cDNA libraries of 
each viruliferous and nonviruliferous \( T. \) palmi population. 
Around 46.84 Gb of the sequence was generated. The average 
reads per library were about 26,021,240 (Supplementary 
Table 2). After preprocessing, a total of 156,074,069 clean reads 
of 20–150 bp were filtered from all six libraries. The preprocessed 
reads ranged between 99.95 and 99.98%. After removing the 
low-quality reads from all the sets, the high-quality reads were 
used for further analyses. These were mapped with the 
\( T. \) palmi genome (Guo et al., 2020) yielding an average mapping 
percent of 97.31%.

The differential expression analysis identified a total of 28,165 
transcripts in the viruliferous vs. nonviruliferous \( T. \) palmi 
populations. Out of these, a total of 2,363 transcripts were 
significantly expressed in viruliferous \( T. \) palmi at stringent 
filtering parameters (log₂ fold change > ±2 and adjusted value 
of \( p < 0.05 \)). A total of 1,383 differentially expressed transcripts 
were upregulated, while 980 transcripts were downregulated 
in viruliferous (pooled TpTrI) as compared to nonviruliferous 
(pooled TpTrH) populations (Figure 1). Table 1 represents 
the top 20 up- and downregulated genes of \( T. \) palmi under 
the stringent filtering criteria in response to GBNV-infection. 
The putative genes of \( T. \) palmi such as cytochrome P450 (CYP), 
gamma-aminobutyric acid (GABA) receptor, heat shock protein 
(hsp70), nephrin, sperm-associated antigen 6 (spa6)-like, and 
ubiquitin-like containing PHD and RING finger domains 1 
(UHRF-1)-binding protein 1, etc. were found highly upregulated 
after exposure to GBNV. Significant downregulations of ABC 
transporter, arrestin domain-containing 3 (ARRDC3), elongation 
of very-long-chain fatty acid (ELOVL), dynamin-1-like, serpin 
H1-like, tyrosine-protein kinase Receptor Tie-1 (TIE1), larval 
cuticle protein A2B-like, pupal cuticle protein C1B-like, and 
endocuticle structural glycoprotein SgAbd-2-like, etc., were 
also observed.

GO and KEGG Pathway Enrichment 
Analyses of Differentially Expressed Genes

The 1,383 (58.52%) up- and 980 (41.48%) downregulated DEGs 
were categorized under the three major classes, namely, cellular 
components, biological processes, and molecular functions. The
highest number of GO terms were categorized into molecular functions (70%) followed by cellular components (24%), and biological processes (6%; Figure 2A). In the molecular functions category, there was an abundance of genes associated with catalytic activity followed by binding, molecular transduction activity, antioxidant activity, and transporter activity. Similarly, genes associated with the membrane, membrane part, cell, cell part, protein-containing complex, organelle, synapse, synapse part were differentially enriched under the cellular components category. In the biological processes, genes involved in the cellular process, response to stimuli, signaling, regulation of the biological process, localization, metabolic process, biological regulation, and signaling were enriched. Within each of the functional groups, the top enrichment terms for both up- and downregulated DEGs identified as per Fisher’s exact test value of \( p \) are listed in Figures 2B, C, respectively.

Kyoto Encyclopedia of Genes and Genomes pathway analysis of DEGs showed that the genes were involved in the functions like metabolic pathways, biosynthesis of secondary metabolites, cellular processes, endocytosis, organismal systems, and signaling pathways. Majorly affected metabolic pathways were glycerolipid, glycerophospholipid, purine, fructose and mannose, galactose, glutathione, and carbon metabolism. The differentially expressed signaling pathways were calcium, cyclic adenosine monophosphate (cAMP), glucagon, hypoxia-inducible factor 1 (HIF-1), mitogen-activated protein kinase (MAPK), neurotrophin signaling, phospholipase D, Ras-proximate-1 (Rap1) signaling, and Ras signaling pathways. The most affected cellular and organismal systems were dopaminergic synapses, endocytosis, glutamatergic synapse, lysine degradation, neuroactive ligand-receptor interaction, oocyte meiosis, and synaptic vesicle cycle. In KEGG pathway enrichment analysis, the highest number of DEGs was annotated in drug metabolism (map000982, 00983) under the class xenobiotics biodegradation and metabolism followed by glycerolipid metabolism (map00561), methane metabolism (map00680), glycerophospholipid metabolism (map00564), and fructose and mannose metabolism (map00051; Figure 3). The pathway enrichment of DEGs showed the DEGs were significantly enriched in ascorbate and aldarate metabolism (map00053), steroid hormone biosynthesis (map00140), and metabolism of xenobiotics by cytochrome P450 (map00980) pathways. The pathways identified in GO enrichment analysis were consistent with the findings of the KEGG pathway study.

**Gene Regulatory Network Analysis**

A total of 40 nodes comprised the top up- and downregulated DEGs were considered in network analysis. The degree and betweenness centrality in the interaction network of the top DEGs were calculated. Interaction networks corresponding with the highly regulated DEGs demonstrated a total of 438 edges. The degree of certain nodes was markedly higher compared with the average degree in the networks (Figure 4). The upregulated DEGs with significantly higher degrees were calphatin-like isoform X2, proline-rich protein 36-like isoform X1, dipeptidyl-aminopeptidase B, sodium channel protein para isoform X8, nephrin-like, cAMP-specific 3’,5’-cyclic phosphodiesterase isoform X1, UHRF1-binding protein 1-like isoform X3, spag6-like, laccase-5, CYP 4C1-like,
| S. No | Name of the gene | Accession | GO term |
|-------|------------------|-----------|---------|
| **Upregulated DEGs** | | | |
| 1 | Calphotin-like isoform X2 | XM_034379643 | Epithelium-like organization (GO:0140509), identical protein binding (GO:0042822), and skein-like inclusion (GO:0097420). Regulate the amount of free cytoplasmic calcium. Rhabdomere development and photoreceptor cell survival (Martin et al., 1998) |
| 2 | Proline-rich protein 36-like isoform X1 | XM_034379634 | Proline-rich region binding (GO:0070064), protein peptidyl-prolyl isomerization (GO:0000413), and ubiquitin-like protein binding (GO:0032182) |
| 3 | LOW-QUALITY PROTEIN; probable dipeptidyl-aminopeptidase B | XM_034387198 | Cytoplasm protein quality control (GO:0140455), aminopeptidase activity (GO:0004177), cysteine-type aminopeptidase activity (GO:0070005), and serine-type aminopeptidase activity (GO:0070009) |
| 4 | Sodium channel protein para isoform X8 | XM_034385472 | Sodium channel activity (GO:0005272), sodium channel complex (GO:0034706), and protein transmembrane transporter activity (GO:00008320) |
| 5 | Nephrin-like | XM_034394430 | Epithelium-like (GO:0140509), skein-like inclusion (GO:0097420), X11-like protein binding (GO:0042882), and Toll-like receptor binding (GO:0035325) |
| 6 | cAMP-specific 3',5'-cyclic phosphodiesterase isoform X1 | XM_034385045 | 3',5'-cyclic-AMP phosphodiesterase activity (GO:0004115), phosphoric diester hydrolase activity (GO:0008081), and 3',5'-cyclic diguanylic acid metabolic process (GO:0052653) |
| 7 | UHRF1-binding protein 1-like isoform X3 | XM_034383841 | X11-like protein binding (GO:0042988), ubiquitin-like protein binding (GO:0032182), and identical protein binding (GO:0042820) |
| 8 | Protein furry-like isoform X1 | XM_034399231 | Identical protein binding (GO:0042820), X11-like protein binding (GO:0042988), ubiquitin-like protein binding (GO:0032182), Sm-like protein family complex (GO:0120114), and fasciclin-like-arabino galactan protein metabolic process (GO:0010498) |
| 9 | SCY1-like protein 2 isoform X3 | XR_004587416 | Identical protein binding (GO:0042820), X11-like protein binding (GO:0042988), ubiquitin-like protein binding (GO:0032182), Toll-like receptor 2 binding (GO:0035663), Sm-like protein family complex (GO:0120114), and Toll-like receptor signaling pathway (GO:0034134) |
| 10 | Gamma-aminobutyric acid receptor subunit beta isoform X8 | XR_004588251 | Gamma-aminobutyric acid receptor clustering (GO:00097112), gamma-aminobutyric acid receptor clustering (GO:0014051), and gamma-aminobutyric acid transport (GO:00115812), GABA receptor complex (GO:1902710), and gamma-aminobutyric acid transmembrane transporter activity (GO:0010498) |
| 11 | Sperm-associated antigen 6-like | XM_034382859 | TAP complex (GO:0042825), sperm motility (GO:0097722), sperm capacitation (GO:0048248), and sperm flagellum (GO:00336126) |
| 12 | Proteoglycan 4-like isoform X4 | XM_034375036 | Proteoglycan binding (GO:0043394), Toll-like receptor 4 binding (GO:0035663), and Toll-like receptor 4 signaling pathway (GO:0034142) |
| 13 | Sodium channel protein para isoform X5 | XM_034385468 | Sodium channel activity (GO:0005272), sodium channel complex (GO:0034706), sodium channel regulator activity (GO:0017080), and regulation of voltage-gated sodium channel activity (GO:1905150) |
| 14 | Laccase-5 | XM_034375390 | Oxidoreductase activity, acting on diphenols and related substances as donors, oxygen as acceptor (GO:0016682), hydroquinone: oxygen oxidoreductase activity (GO:0032716), MDA-5 binding (GO:00358556), laminin-5 complex (GO:0005610), and rhombomere 5 development (GO:0021571) |
| 15 | Cytochrome P450 4C1-like | XM_034387022 | Retinoic acid 4-hydroxylase activity (GO:0008401), arachidonic acid (11,12-epoxygenase activity (GO:0008405), and coumarin 7-hydroxylase activity (GO:0008389) |
| 16 | Serine protease snake-like | XM_034378873 | Serine-type peptidase activity (GO:0035663), serine protease inhibitor activity (GO:0035663), and serine protease inhibitor activity (GO:0035663) |
| 17 | Phosphoribosylformylglycinamidine synthase | XM_034387894 | Serine-type peptidase activity (GO:0007839), cysteine-type peptidase activity (GO:0070005), and serine-type peptidase activity (GO:0070005) |
| 18 | Lipase 3-like isoform X2 | XM_034393741 | Phosphoribosylformylglycinamidine synthase activity (GO:0004642), phosphoribosylformylglycinamidine cyclo-ligase activity (GO:0008441), and hercyryl selenocysteine synthase (GO:0044876) |
| 19 | Heat shock 70kDa protein II-like | XM_034395223 | Lipase activity (GO:0016298), lipase binding (GO:0035473), toll-like receptor 3 signaling pathway (GO:0034138), and regulation of lipase activity (GO:0060191) |

(Continued)
| S. No | Name of the gene | Accession | GO term |
|------|----------------|-----------|---------|
|      | **Fatty acid synthase-like** | XM_034388838 | Fatty acid synthase complex (GO:0005835), fatty acid synthase activity (GO:0004312), fatty acid binding (GO:0008504), fatty acid elongation (GO:0030497), and fatty acid homeostasis (GO:0005089) |
|      | **Proline-rich protein Haelli subfamily 1-like** | XM_034391864 | Proline-rich region binding (GO:0070064), intramolecular proline-rich ligand binding (GO:0032840), ficolin-1-rich granule (GO:0101002), and protein peptidyl-prolyl isomerization (GO:0004143) |
|      | **Arrestin domain-containing protein 3-like** | XM_034389930 | Arrestin family protein binding (GO:1990763), protein-containing complex (GO:0032391), RING-like zinc finger domain binding (GO:0071535), and RING protein domain binding (GO:00059719) |
|      | **Mucin-2-like isoform X2** | XM_034396273 | Mucin granule (GO:0098594), Toll-like receptor 2 binding (GO:0035663), and toll-like receptor 2 signaling pathway (GO:0034134) |
|      | **Basic salivary proline-rich protein 4-like** | XM_034378770 | Proline-rich region binding (GO:0070064), intramolecular proline-rich ligand binding (GO:0032840), protein peptidyl-prolyl isomerization (GO:00004143), and procollogen-proline 4-dioxigenase complex GO:0016222 |
|      | **Elongation of very-long-chain fatty acids protein AAEL008004-like** | XM_034386603 | Very long-chain fatty acid catabolic process (GO:0002760), very long-chain fatty acid biosynthetic process (GO:0042761), and very long-chain fatty acid omega-hydroxylase activity (GO:0146662) |
|      | **Ubiquitin carboxyl-terminal hydrolase 36-like isoform X1** | XR_004588219 | Ubiquitin-like hydrolase activity (GO:0140491), ubiquitin-like protein binding (GO:0032182), and ubiquitin-like protein transferase activity (GO:0019787) |
|      | **Serine/arginine repetitive matrix protein 1-like** | XM_034375486 | Structural constituent of virion (GO:0039660), protein-arginine deiminase activity (GO:0044688), protein arginine phosphatase activity (GO:00098627), and protein arginine kinase activity (GO:1990424) |
|      | **Serpin H1-like** | XM_034391757 | H1 histamine receptor binding (GO:0031807), serpin family protein binding (GO:0097854), epithelium-like organization (GO:0104509), and skin-inclusion complex (GO:0097420) |
|      | **Myrosinase 1-like** | XM_034376507 | Thioglucoisidase binding (GO:0010190), thioglucoisidase complex 1 (GO:0010169), toll-like receptor 1 signaling pathway (GO:0034130), and glucagon-like peptide 1 receptor activity (GO:0044508) |
|      | **Fidgetin-like protein 1 isoform X1** | XM_034396404 | Toll-like receptor 1-Toll-like receptor 2 protein complex (GO:0035354), toll-like receptor 1 signaling pathway (GO:0034130), and glucagon-like peptide 1 receptor activity (GO:0044508) |
|      | **ABC transporter F family member 4-like** | XM_034380645 | Sm-like protein family complex (GO:0120114), ABC-type transporter activity (GO:0140359) |
|      | **Peroxiredoxin-6-like** | XM_034396273 | Peroxiredoxin activity (GO:0051920), toll-like receptor 6 signaling pathway (GO:0034150), and laminin-6 complex (GO:0005611) |
|      | **Alpha-tocopherol transfer protein-like** | XM_034375040 | Alpha-tocopherol omega-hydroxylase activity (GO:0032871), vitamin E binding (GO:0008431), tocopherol cyclase activity (GO:0009798), and genetic transfer (GO:0009292) |
|      | **Tyrosine-protein kinase receptor Tie-1-like** | XM_034386734 | Transmembrane receptor protein tyrosine kinase activity (GO:0004714), protein tyrosine kinase catalytic subunit activity (GO:0038062), and transmembrane receptor protein tyrosine kinase signaling pathway (GO:0007169) |
|      | **Dynamin-1-like protein** | XM_034384104 | DNM1L-mediated stimulation of mitophagy in response to mitochondrial depolarization (GO:00061735), Toll-like receptor 1-Toll-like receptor 2 protein complex (GO:0035354), and dynamin family protein polymerization involved in membrane fission (GO:0003373) |
|      | **Larval cuticle protein A2B-like** | XM_034397437 | Structural constituent of chitin-based larval cuticle (GO:0008010), larval cuticle development (GO:0008363), larval serum protein complex (GO:0005616), larval behavior (GO:0030497), and A2B adenosine receptor binding (GO:0031688) |
|      | **LOW-QUALITY PROTEIN: pupal cuticle protein C1B-like** | XM_034378539 | Cytoplasm protein quality control (GO:0140455), pupal chitin-based cuticle development (GO:0008364), structural constituent of pupal chitin-based cuticle (GO:0008601), and pupal development (GO:0035208) |
|      | **Serine protease stubble-like** | XM_034381350 | Proteinase activated receptor binding (GO:0031688), serine binding (GO:0070505), and serine transport (GO:0032229) |
|      | **Endocuticle structural glycoprotein SgAbd-2-like** | XM_034395161 | Glycoprotein complex (GO:00090665), glycoprotein transport (GO:0034436), Toll-like receptor 2 binding (GO:0035663), N-glycan fucosylation (GO:0038671), protein deglycosylation (GO:0006517), and response to glycoprotein (GO:1904587) |
|      | **Phenoloxidase 2-like** | XM_034391057 | Toll-like receptor 2 binding (GO:0035663), toll-like receptor 2 signaling pathway (GO:0034134) |
phosphoribosylformylglycinamidine synthase (PFAS), and hsp70 II-like. Among the downregulated nodes, a group of DEGs associated with cuticle development such as larval cuticle protein A2B-like, endocuticle structural glycoprotein SgAbd-2-like, and ABC transporter F family member 4-like were with higher degrees of interactions. Besides, proline-rich protein HaeIII subfamily 1-like, ARRDC3, serpin H1-like, TIE-1-like, myrosinase 1-like, alpha-tocopherol transfer protein (TTPA)-like, and dynamin-1-like protein showed significant interactions with higher degrees.

Validation of DEGs in RT-qPCR
To validate the differential gene expression data, thirteen highly expressed genes of T. palmi were selected and mRNA expression levels were quantified in RT-qPCR in response to GBNV infection. A part of the samples used for RNA-Seq was preserved and used in RT-qPCR assay. Primer pairs designed for this study were initially optimized in a gradient PCR (Supplementary Table 1). Primer pairs of target genes that produced a single sharp amplicon at the same PCR conditions for endogenous control (β-tubulin) primers were selected for RT-qPCR assay. The primer pair specific to β-tubulin produced sharp bands at all annealing temperatures between 53 and 59°C. The annealing temperature of primer pairs for each target gene was standardized within the same temperature range. One best primer pair for each of the target genes was optimized for RT-qPCR assay. The annealing temperature, amplicon size, and melting temperature of each amplicon in RT-qPCR are included in Supplementary Table 1. The primer pairs for target and endogenous control genes produced single specific peaks without any secondary amplification in the RT-qPCR melting curve analysis that indicated the specificity of the reactions (Supplementary Figure 1).

The relative expression of target genes was estimated by a 2−ΔΔCT method. The variation among the biological replicates was normalized with respect to the Ct value of endogenous control, β-tubulin. Among the selected highly expressed genes, the expression of UHRFI-binding protein 1 of T. palmi was upregulated by log2 5.5-fold in RT-qPCR in response to GBNV infection (Figure 5). Likewise, mRNA expression of nephrin was upregulated by log2 4.8-fold in viruliferous T. palmi adults in comparison to nonviruliferous adults. mRNA levels of spag6-like, hsp70, and GABA receptor were upregulated by log2 3.37, 0.98, and 0.80-fold, respectively, post-GBNV exposure. In viruliferous T. palmi, the expression of serpin was downregulated by log2 8.5-fold. Similarly, log2 4.3-fold downregulation of tyrosin kinase was recorded in RT-qPCR in response to GBNV infection. Expression of ABC transporter F family member 4-like gene was also downregulated by log2 3.57-fold, while it was log2 1.65-fold downregulations for ARRDC3 in viruliferous T. palmi. Some other genes such as elongation of fatty acid chain, dynamin-1-like, myrosinase 1-like showed log2 1.36, 0.66, and 2.57-fold, respectively, lower expression in viruliferous T. palmi as compared to nonviruliferous.

The fold change values estimated from RNA-Seq were plotted against fold change values obtained in RT-qPCR assays of the selected genes (Figure 5). The Pearson’s correlation coefficient of 0.81 indicated the acceptability of DEGs through RNA-Seq (Supplementary Table 3).
DISCUSSION

Multiplication of GBNV in *T. palmi* suggests a potential alteration in the physiological process of *T. palmi*. Pathogenic effects of GBNV are evident by reduced survival and adult longevity of *T. palmi* (Ghosh et al., 2019). The fecundity of *T. palmi* decreases sharply after GBNV exposure. GBNV infection in *T. palmi* also induces more females in progenies (Ghosh et al., 2019). We observed the same trend in the biological traits of *T. palmi* post-GBNV exposure in the current study. In thrips–tospovirus relationships, adults of thrips are vector competent only if the virus is acquired during the larval stages (Wetering et al., 1996; Mou et al., 2021). Only during the early larval stage, the virus is allowed to infect the midgut (Ullman et al., 1992; Ohnishi et al., 2001). The virus infects the epithelial cells of the anterior midgut of *T. palmi* and then progresses to the primary salivary glands through a pair of connecting ligaments (Ghosh et al., 2021). The tospovirus infection in adults of *T. palmi* is retained in visceral and longitudinal muscles of the midgut. Tospovirus may not be able to infect the epithelial cells of adult thrips (Ghosh et al., 2021). A 50-kDa protein receptor present on the epithelial cells of thrips is abundant during the larval stage only (Bandla et al., 1998). The tospovirus propagates in the midgut and salivary glands of *T. palmi* (Ghosh et al., 2021; Mou et al., 2021). In thrips, a transovarial infection barrier has been discovered, which prevents the tospovirus from transmitting vertically (Wijkamp et al., 1996; Mou et al., 2021). The next generation of thrips again needs to acquire the virus during their early larval stage.

Manipulation of insect vectors by plant viruses is common in thrips–tospovirus systems (Deangelis et al., 1993; Wijkamp et al., 1996; Belliure et al., 2008; Ingwell et al., 2012; Shrestha et al., 2012; Ghosh et al., 2019). The central hypothesis of the present study tells that the successful GBNV transmission by viruliferous *T. palmi* perturbs the expression of candidate genes associated with innate immunity, cuticle development, cellular receptor binding, and signaling of its vector. The differential transcriptomic response of *T. palmi* induced by GBNV infection was distinguished by RNA-Seq. KEGG analyses further explained several molecular pathways involved in *T. palmi*-GBNV interactions. Cluster analysis of expressed genes from viruliferous and nonviruliferous *T. palmi* revealed that 1,383 and 980 genes of *T. palmi* were up- and downregulated, respectively in response to GBNV infection. TSWV infection regulated expression of

![Figure 3](https://example.com/figure3.png)
FIGURE 4 | Gene regulatory network (GRN) of DEGs. Red squares represent upregulated DEGs, and blue squares represent downregulated DEGs.

FIGURE 5 | Expression of Thrips palmi putative genes in response to GBNV infection in RNA-Seq and reverse transcriptase-quantitative real-time PCR (RT-qPCR). The values of log2-fold changes calculated in RNA-Seq analysis were in accordance with the RT-qPCR fold change values. Pearson’s correlation coefficient value was 0.81 as calculated using the CORREL function in MS Excel.
127 transcripts in *F. occidentalis* adults. Expression of genes like arylphorin, chitinase, hemocyanin, and hexamerin was modulated in *F. occidentalis* adults in response to TSWV infection (Schneweis et al., 2017). A total of 562 contigs were differentially regulated by TSWV in adults of *F. fusca*. Reproduction, embryo development, and growth-related pathways were identified with upregulated contigs in virus-exposed adults (Shrestha et al., 2017). In the present study, candidate genes, such as CYP, GABA receptor, hsp70, laccase 5, nephrin, spag6, PFAS, proteoglycan, and UHRF1-binding protein 1 associated with innate immune response, receptor binding, signaling, endocytosis, viral replication, and apoptosis (Qin et al., 2009; González-Santoyo and Córdoba-Aguilar, 2012; Götz et al., 2012; Cooley et al., 2016; Zhang et al., 2016; Zhu et al., 2017; Lu et al., 2019) were upregulated in *T. palmi* adults in response to GBNV infection. A group of genes associated with host innate immune and stress response, such as hsp70, UHRF1-binding protein 1, spag6, laccase-5, and PFAS was upregulated in viruliferous *T. palmi* (Zhao and Jones, 2012; Cooley et al., 2016; Zhang et al., 2016; Chen et al., 2020). *Hsp* belongs to a class of functionally related proteins involved in the folding and unfolding of other proteins. This group of proteins is elevated in insect cells in response to various biotic and abiotic stresses (Zhao and Jones, 2012). Besides, hsp70 is an active regulator of tomato yellow leaf curl virus (TYLCV) infection in its vector, *Bemisia tabaci* (Götz et al., 2012). In infected host cells, hsp70 interacts with TYLCV coat protein (CP) and is involved in the translocation of TYLCV CP from the cytoplasm to nucleus (Götz et al., 2012; Gorovits and Czosnek, 2013, 2017). Upregulations of *T. palmi* hsp70 were also recorded in response to CaCV infection (Gamage et al., 2018). However, interaction of hsp70 with GBNV surface proteins needs further experimental evidence. *UHRF1-binding protein 1* is best known for its function in maintaining DNA methylation and acting as an epigenetic regulator. When the host is attacked by pathogens, gene expression levels are regulated by a DNA methylation pattern that leads to activation or suppression of several signaling pathways and triggers a series of immune response events against viral invasion (Zhang et al., 2016). Thus, upregulation of *UHRF1-binding protein 1* in viruliferous *T. palmi* adults suggests its role in innate immunity. Upregulation of spag6 in the present study might also be due to the immune response of *T. palmi* to GBNV infection. The role of mouse *spag6* in immune response was described by Cooley et al. (2016). In insects, laccase is abundant in the cuticle and has been involved in cuticle sclerotization (Hattori et al., 2010). Laccase 5 was significantly upregulated in viruliferous *T. palmi*. Laccases include phenol oxidases (Janusz et al., 2020) that are activated in response to viral infection and oxidative stress (González-Santoyo and Córdoba-Aguilar, 2012; Chen et al., 2020). A similar response of *T. palmi* laccase post-CaCV infection (Gamage et al., 2018) suggests its association in maintaining oxidative stress due to tospovirus infection in *T. palmi*. In consistent with the previous report of CaCV-exposed *T. palmi* (Gamage et al., 2018), PFAS was also upregulated in *T. palmi* in response to GBNV infection. PFAS was also found to be upregulated in *Culex quinquefasciatus* after infection with West Nile virus (Girard et al., 2010). PFAS not only plays a role in the immune escape process of the virus but also has a crucial impact on other pathological processes (Lu et al., 2019). Pathological effects such as reduced survival and adult longevity of *T. palmi* were also recorded post-GBNV exposure in the present study. The role of PFAS in triggering such pathological effects in *T. palmi* may be worth for further investigation.

The GBNV is supposed to be internalized in *T. palmi* cells by clathrin-mediated endocytosis (CME; Jagdale and Ghosh, 2019). DEGs, such as nephrin, proteoglycan 4-like, and heparin sulfate proteoglycan (HSPG) associated with endocytosis and entry receptors (Qin et al., 2009; Christianson and Beltin, 2014; Cagno et al., 2019) were upregulated upon GBNV infection. *Nephrin* is internalized during the signal transduction pathway of raft-mediated endocytosis and classical CME (Qin et al., 2009). The upregulation of nephrin in the present study indicated its involvement in CME of the GBNV proteins in *T. palmi* cells. Proteoglycan 4-like, upregulated in *T. palmi* post-GBNV infection, might be associated with receptor binding and entry of tospovirus in thrips. HSPG is a cell-surface endocytosis receptor and is involved in cellular interactions with several human viruses. The interaction with HSPG is used by viruses to increase their concentration at the cell surface and augment their chances of binding more specific entry receptors (Cagno et al., 2019). HSPG is an integral factor for the attachment of Arbovirus to the salivary gland duct in mosquitoes (Ciano et al., 2014).

In insects, GABA receptors are found throughout the central nervous system and are reported to be an important target site for several insecticides (Anthony et al., 1993). In mosquitoes, GABA receptor was associated with viral replication (Zhu et al., 2017). CYP imparts resistance against insecticides in *F. occidentalis* and *T. tabaci* (Zhang et al., 2013; Rosen et al., 2021). In the present study, upregulations of GABA receptor and CYP in viruliferous *T. palmi* were in accordance with the response of CaCV-exposed *T. palmi* (Gamage et al., 2018). CYP-mediated metabolism of xenobiotics pathway was also enriched in viruliferous *T. palmi*. An in-depth study of the mechanism involved would be necessary to shed further light on the role of GABA receptor and CYP in tospovirus replication in *T. palmi*.

A considerable number of *T. palmi* genes were under-regulated in response to GBNV infection. Downregulations of genes like ABC transporter, ARRDC3, cuticular proteins, dynamin-1-like protein, myrosinase-1-like, and serpin facilitate the circulation and multiplication of GBNV in *T. palmi*. Several cuticular protein genes were downregulated in transcriptomic studies of TSWV-infected *F. occidentalis* (Schneweis et al., 2017) and CaCV-infected *T. palmi* (Gamage et al., 2018). In the present study, downregulations of *T. palmi* larval cuticle protein A2B-like, papul cuticle protein C1B-like, endocuticle structural glycoprotein SgAbd-2-like were recorded in response to GBNV infection. Endocuticle structural glycoprotein localizes in the midguts and salivary glands of *F. occidentalis* (Schneweis et al., 2017). *ABC transporter* has also been reported to be involved in molting and cuticle formation in insects (Broehan et al., 2013) and was downregulated in the present study. Virus infection suppresses the activities of cuticular proteins embedded in the peritrophic matrix, a structural barrier to pathogen attack (Kato et al.,...
In consistent with the downregulation of genes associated with cuticle development, a delayed molting of the larval instars post-GBNV exposure was recorded in the present study. Delayed molting of viruliferous T. palmi was earlier reported by Ghosh et al. (2019). Under-expression of cuticular, endo-cuticular proteins, and ABC transporter of T. palmi gives an advantage of the delayed molting and membrane dysfunction for virus infection in the thrips gut. However, insect cuticle-related contigs were upregulated in TSWV-infected E. fusca (Shrestha et al., 2017). The innate differences among the thrips species, experimental setup, and virus isolates could be the reason behind the variation in response.

A few genes associated with innate immune responses like serpins and dynamin were also downregulated in T. palmi in response to GBNV infection. Insect innate immunity is regulated by serpins by inhibiting serine proteinase cascades that instigate innate immune responses. Serpins could also possess direct anti-pathogen activity upon infection (Levashina et al., 1999). Dynamin-1-like protein is close relative to dynamin and has a role in fission of mitochondrial and peroxisomal membranes. In mammals, mitochondrial dynamin affects disease resistance. Pathogens have evolved several strategies to interfere with mitochondrial dynamins to suppress the immune responses (Spier et al., 2019). Several pathogens directly target the dynamin-related protein (DRP1) to block the innate immune responses (Suzuki et al., 2014; Yu et al., 2015). Downregulation of dynamin-1-like protein and serpin H1-like genes in viruliferous T. palmi help in evading innate immune responses in T. palmi to favor the virus infection and multiplication in vector cells. Gene like ELOVL was downregulated in viruliferous T. palmi. Accumulation of ELOVL fatty acids in the cells leads to necroptosis (Parisi et al., 2017). Inhibition of ELOVL prevents the loss of plasma membrane integrity and cell death. Downregulation of ELOVL gene in T. palmi helps maintain the integrity of cells and supports viral growth.

A group of genes associated with cell surface receptor and cellular transport, such as TIE1, ARRD3C, TTPA-like, and potassium channel subfamily K member 18 (KCNK18)-like was regulated in T. palmi in response to GBNV infection. TIE1 is the major class of enzyme-coupled cell surface receptors activated in response to external signals such as growth factors, cytokines, and hormones. Host signaling through tyrosine receptor kinases has also been found to play a key role in virus replication (Kumar et al., 2011). Recently, it has been demonstrated that tyrosine kinases are required by viruses for intracellular entry (Stewart et al., 2021). ARRD3C is a member of the family of α-arrestins in the superfamily of arrestin adaptor proteins (Patwari and Lee, 2012). The α-arrestin, ARRD3C mediates ALIX ubiquitination and G protein-coupled receptor lysosomal sorting (Dores et al., 2015). Ubiquitination of ALIX has also been observed during the budding of viruses from the plasma membrane in mammals (Sette et al., 2010). TTPA-like and KCNK18-like that encode proteins associated with inter- and intracellular transport were downregulated in response to GBNV infection. Similar results were recorded by Gamage et al. (2018). TTPA isoform 1 and 2, and KCNK9 were also downregulated in response to TSWV infection in E. fusca (Shrestha et al., 2017). The requirement of potassium channels for infecting the host cell by bunyaviruses was reported by Hover et al. (2016). The antiviral activity of the potassium ion channel in honeybees was reported by O’Neal et al. (2017). However, the molecular mechanisms underlying the downregulation of these genes in response to GBNV infection are not known and need in-depth study.

The comparison of the DEGs reported in CaCV-exposed T. palmi (Gamage et al., 2018) with our work showed 103 functions in common (Supplementary Figure 2). However, we could find 2,260 genes uniquely expressed in the GBNV-exposed T. palmi, while 347 genes were unique to the CaCV-exposed T. palmi. Among the DEGs, the responses of cuticular proteins, CYP, endocuticle structural glycoprotein, GABA receptor, lsp70, laccase 5, KCNK-like, and TTPA were conserved in both GBNV and CaCV infection but distinct to TSWV infection in F. occidentalis (Schneweis et al., 2017). These candidate genes would be potential targets for novel generic pest control.

Among the major pathways of T. palmi affected by GBNV, metabolic pathways, biosynthesis of secondary metabolites, endocytosis, and signaling pathways were important. Ascorbate and aldarate metabolisms, steroid hormone biosynthesis, cytochrome p 450, and other enzymes-mediated metabolism of xenobiotics, fructose, and mannose metabolism were highly enriched. GRN analysis showed that DEGs associated with innate immune response, cuticle development, receptor signaling, and endocytotic pathways are involved in possible manipulation in host invasion by GBNV.

In conclusion, we have assembled a whole-body transcriptome of adult T. palmi and reported the DEGs in response to GBNV infection. The majority of DEGs are involved in innate immunity, receptor binding and signaling, cuticle development, and endocytosis that facilitate the invasion, circulation, and multiplication of GBNV in T. palmi. The candidate genes of T. palmi that are highly regulated in response to GBNV infection would be potential targets for the effective management of tospoviruses. In addition, data generated in this study will enrich genomic information of thrips and will enable functional studies.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/, PRJNA758768.

AUTHOR CONTRIBUTIONS

AG, VB, VK, and SC conceived and designed the research. DM prepared biological samples. DM, SJn, and Priti carried out the wet laboratory experiments and wrote the draft manuscript. Priti, MI, and SJi analyzed RNA-Seq data. AG and VB reviewed the results and edited the final manuscript. All authors read and approved the manuscript.
FUNDING
The scholarship of DM was supported by Indian Council of Agricultural Research.

ACKNOWLEDGMENTS
This is a part of thesis work of DM submitted to Post Graduate School, IARI, New Delhi. The initial virus culture was supplied by Basavaraj YB (IARI). The authors are thankful to Heena (IARI) for her help in maintenance of virus and insect culture.

SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.773238/full#supplementary-material
Ullman, D., Cho, J. J., Mau, R. F. L., Hunter, W. B., Westcot, D. M., and Custer, D. M. (1992). "Thrips-tomato spotted wilt virus interactions: morphological, behavioral and cellular components influencing thrips transmission," in Advances in Disease Vector Research 9. ed. K. I. Harris (New York: Springer), 195–240.

Ullman, D., Meideros, R., Campbell, L. R., Whitfield, A. E., Sherwood, J. L., and German, T. L. (2002). Thrips as vectors of tospoviruses. Adv. Bot. Res. 36, 113–140. doi: 10.1016/S0065-2296(02)36061-0

Ullman, D., Sherwood, J., and German, T. L. (1997). "Thrips as vectors of plant pathogens," in Thrips as Crop Pests. ed. T. Lewis (Wallingford, UK: CAB International), 539–565.

Walker, A. K. (1992). "Pest status," in Thrips palmi: A Literature Survey, International Institute of Biological Control. ed. D. J. Girling (Ascot, Berks, UK: International Institute of Biological Control), 3–6.

Wetering, F., Goldbach, R., and Peters, D. (1996). Tomato spotted wilt virus ingestion by first instar larvae of Frankliniella occidentalis is a prerequisite for transmission. Phytopathology 86, 900–905. doi: 10.1094/Phyto-86-900

Whitfield, A. E., Ullman, D. E., and German, T. L. (2005). Tospovirus-thrips interactions. Annu. Rev. Phytopathol. 43, 459–489. doi: 10.1146/annurev.phyto.43.040204.140017

Gamage, S. M. K. W., Rotenberg, D., Schneweis, D. J., Tsai, C. W., and Dietzgen, R. G. (2018). Transcriptome-wide responses of adult melon thrips (Thrips palmi) associated with capsicum chlorosis virus infection. PLoS One 13:e0208538. doi: 10.1371/journal.pone.0208538

Wijkamp, I., Goldbach, R., and Peters, D. (1996). Propagation of tomato spotted wilt virus in Frankliniella occidentalis does neither result in pathological effects nor in transovarial passage of the virus. Entomol. Exp. Appl. 81, 285–292. doi: 10.1046/j.1570-7458.1996.00098.x

Yu, C. Y., Liang, J. Ji., Li, J. K., Lee, Y. L., Chang, B. L., Su, C. I., et al. (2015). Dengue virus impairs mitochondrial fusion by cleaving mitofusins. PLoS Pathog. 11:e1005350. doi: 10.1371/journal.ppat.1005350

Zhang, Y., Schöttker, B., Florath, I., Stock, C., Butterbach, K., Holleczek, B., et al. (2016). Smoking-associated DNA methylation biomarkers and their predictive value for all-cause and cardiovascular mortality. Environ. Health Perspect. 124, 67–74. doi: 10.1289/ehp.1409020

Zhang, Z., Zhang, C., Li, W., Zhang, J., Huang, E., Yang, J., et al. (2013). De novo transcriptome sequencing in Frankliniella occidentalis to identify genes involved in plant virus transmission and insecticide resistance. Genomics 101, 296–305. doi: 10.1016/j.ygeno.2013.02.005

Zhu, Y., and Jones, W. A. (2012). Expression of heat shock protein genes in insect stress responses. Invertebr. Surviv. J. 9, 93–101.

Zhu, Y., Zhang, R., Zhang, B., Zhao, T., Wang, P., Liang, G., et al. (2017). Blood meal acquisition enhances arbovirus replication in mosquitoes through activation of the GABAergic system. Nat. Commun. 8:1262. doi: 10.1038/s41467-017-01244-6

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Mahanta, Jangra, Priti, Ghosh, Iquebal, Jaiswal, Baranwal, Kalia and Chander. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.