In silico analyses of molecular interactions between groundnut bud necrosis virus and its vector, *Thrips palmi*

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Abstract Groundnut bud necrosis virus (GBNV) is an economically important tospovirus transmitted by *Thrips palmi* (Thysanoptera: Thripidae). The current understanding of thrips-tospovirus interactions is largely based on the tomato spotted wilt virus-*Frankliniella occidentalis* relationship. Only limited information is available for the GBNV-*T. palmi* system. In the present study, available genome data of *T. palmi* and GBNV were used to predict the protein partners that may play a crucial role in the internalization of GBNV virions into thrips cells. Computational analyses showed that the GBNV precursor glycoprotein bears a signal peptide of 24 amino acids and a secondary cleavage site at position 434–435 separates the amino-terminal mature glycoprotein (G_N) from the carboxyl-terminal glycoprotein (G_C). Potential interactions of GBNV glycoproteins were predicted with *T. palmi* enolase, cathepsin, C-type lectin, clathrin and vacuolar ATP synthase subunit E. The in silico analyses suggested that C-type lectin is the primary cellular receptor to interact with GBNV-G_N. After receptor binding, virus particles probably enter vector cells by clathrin-mediated endocytosis. This is the first in silico evidence of GBNV-*T. palmi* protein interaction.

Keywords Melon thrips · GBNV · Modeling · Docking · Virus-vector relationship · Protein–protein interaction

Introduction

Groundnut bud necrosis virus (GBNV; genus *Tospovirus*, family *Peribunyaviridae*, order *Bunyavirales*) is one of the predominant plant viruses causing substantial losses of agricultural produce worldwide. GBNV accounts for losses of US\$ 89 million annually with 70–90% yield loss in groundnut and 30% in potato [29, 22]. Infection of GBNV causes mild chlorotic spots on young quadrifoliate leaves, and subsequently necrosis and chlorotic rings develop. Secondary symptoms include stunting, axillary shoot proliferation, and malformation of leaflets. *Thrips palmi* (Thysanoptera: Thripidae) is known to vector GBNV [40, 9]. Not much is known about the relationships of thrips and tospoviruses. Based on the well-studied tomato spotted wilt virus (TSWV)-*Frankliniella occidentalis* relationship, it is understood that virus propagates in the thrips body and is transported through the midgut to the salivary gland [38, 39, 43, 44]. Virus transmission occurs exclusively by adult thrips while virus acquisition takes place during the early larval stages [30]. Differential transcriptomics and proteomics of *F. occidentalis* in response to TSWV have predicted few probable receptors for TSWV [3, 33]. The glycoprotein G_N of TSWV is involved in the primary binding to the thrips midgut epithelial cells, whereas G_C is necessary for fusion [42–44, 7]. But no such study has been undertaken to unveil the interaction between GBNV and *T. palmi*. The primary reason for this was a lack of structural data of GBNV proteins and receptors of *T. palmi*. In the present study, we have utilized the available data resources to understand the protein–protein interactions between

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GBNV and *T. palmi* and interpreted the cellular entry mechanism of GBNV in *T. palmi*. This will be helpful to better understand the relationship of GBNV-*T. palmi* and to devise strategies to restrict the spread of GBNV by *T. palmi*.

Material and methods

GBNV glycoprotein analyses

The complete genome sequence of GBNV available from NCBI (accession: PRJNA14766) was utilized in this study. The glycoprotein precursor sequence (GPp) was obtained from NCBI protein database (accession: NP_619703). The presence of a signal peptide was identified using SignalP 4.1 server [26]. N- and O-linked glycosylation were predicted by NetNGlyc 1.0 (<http://www.cbs.dtu.dk/services/NetNGlyc/>) and NetOGlyc 4.0 [35]. Transmembrane domains were recognized using TMHMM, HMMTOP, and TmPred [17, 37, 12].

Retrieval of *T. palmi* protein sequences

A single non-annotated shotgun transcriptome is available on NCBI TSA database (accession: PRJNA219609) [25]. The *T. palmi* proteins most likely to interact with GBNV were shortlisted based on information available from the interaction of animal-infecting bunyaviruses with their hosts. The gene sequences were retrieved from the whole transcriptome of *T. palmi* based on sequence similarity with *Drosophila melanogaster*. The coding sequences were determined by NCBI ORF finder [41] and submitted to ExPASy translate [8] to obtain the putative protein sequences.

Modeling of proteins, analyses, and refinement

T. palmi proteins were modeled using MODELLER9.16 [31]. As no structural homologs were found for GBNV glycoproteins, ab initio modeling was done using I-TASSER [48]. The best model was used as a template for modeling the same protein with MODELLER9.16. The PDB files obtained from MODELLER9.16 were then optimized using Swiss PDB (SPDB) viewer [10] energy optimization. Ramachandran plot of the modeled proteins was generated by RAMPAGE (<http://mordred.bioc.cam.ac.uk/~rapper/rampage.php>). ProSA [46] was used to obtain overall protein quality, Z-score, errors in three-dimensional structures of proteins and the energy graph of the structure. Based on the RAMPAGE and ProSA analyses, the proteins with some errors in their structures were subjected to the loop refinement algorithm of MODELLER9.16. Chiron

portal [28] and energy minimization algorithm of SPDB viewer were used for further energy minimization.

Protein–protein docking of probable interacting partners

Probable interactions between GBNV glycoproteins and *T. palmi* proteins were recognized using PatchDock [5, 32]. The results were further refined using FireDock [2, 24]. Global score values were normalized by calculating the Z-score using the formula $Z = (x - \mu)/\sigma$, where x is the global score, μ is the average global score and σ is the standard deviation [14]. These results were analyzed using the statistical tools (average, standard deviation, standardization, and rank analysis) in Microsoft Excel 2013. The three best results were considered for site-specific docking in Z-dock server [27]. The interfacial interactions and Gibbs free energy values were recorded using PDBePISA [6, 15, 16].

Results

GBNV glycoprotein sequence

GBNV GPp sequence retrieved from NCBI had 1121 amino acids. SignalP4.1 recognized the presence of a signal peptide from amino acids 1 to 24 (Fig. 1). A secondary cleavage site at position 434–435 was predicted to separate the amino terminus (G_N) from carboxyl-terminus (G_C) of the glycoprotein. Potential N-linked glycosylation sites

Fig. 1 Analysis of GBNV glycoprotein cleavage sites by SignalP4.1: SignalP4.1 detected the presence of a signal peptide from position 1 to 24 along with a secondary cleavage at position 434 which separates G_N from G_C . The pink line in the middle represents the threshold value. Mean S score and D-score represent the complete stretch of signal peptide. Secondary cleavage at position 434 is represented by max C-score

were observed at amino acids 291, 546, 927 and 1098 (Fig. 2). This predicted N-linked glycosylation was followed at asparagine in the Asn-Xaa-Ser/Thr/Cys stretch where Xaa was any amino acid except proline. Any potential score above 0.5 threshold in NetNGlyc 4.0 was considered to be N-glycosylated. This potential score was based on the analysis by 9 neural networks. The jury agreement indicated the number of neural networks supporting the prediction (Fig. 2). Potential mucin-type O-glycosylation was observed at positions 461 and 599. Any glycosylation site with prediction score above 0.5 was considered to be O-glycosylated. Transmembrane domains were anticipated using the sequence without the signal peptide (Fig. 2). This analysis was carried out considering the hydrophobic nature of the protein domains. Four transmembrane domains were predicted (three in HMMTOP) in GPp (Fig. 2). Several domains of low to medium hydrophobicity were observed in G_C.

Modeling of GBNV glycoproteins

For ab initio modeling of GBNV glycoproteins, five models were predicted by the I-TASSER server. The best protein models for G_N and G_C were selected for further analysis based on C-score, estimated TM-score and estimated RMSD (Table 1). In a benchmark set of 500 non-homologous proteins, C-score of GBNV G_N was highly correlated with TM-score and RMSD. Following energy optimization by SPDBv and refinement, Ramachandran plot of the refined G_N protein model displayed 88.7% in the favorable region and allowed region, and 11.3% in outlier region. Whereas 93.8% of residues were located in favored and allowed regions in case of G_C model. Theoretically predicted models of GBNV glycoproteins were virtually acceptable after refinement.

Modeling of *Thrips palmi* proteins predicted to interact with GBNV glycoproteins

Based on the available information for animal-infecting bunyavirus-host interactions and TSWV- *F. occidentalis*

Fig. 2 Detection of glycosylation sites and transmembrane domains of GBNV precursor glycoprotein. **a** Sequences of N-linked glycosylation with their potential score and jury judgement obtained from NetNGlyc 4.0. Asparagine residues (green line) at positions 291, 546, 927, and 1098 show N-linked glycosylation with the potential more than the threshold value of 0.500 (red line). **b** Positions of Mucin type O-glycosylation were obtained from NetOGlyc1.0. Potential mucin-

type O-glycosylation was observed at positions 461 and 599. Any glycosylation site with prediction score above 0.5 was considered to be O-glycosylated. **c** Transmembrane domains of GBNV GPp were predicted using TMHMM, HMMTOP and TmPred. Four transmembrane domains were predicted in precursor glycoprotein by TMHMM and TmPred, whereas three transmembrane domains were identified by HMMTOP

Table 1 Quality of the GBNV-glycoprotein models obtained by I-TASSER

GBNV glycoprotein	G _N	G _C
C-score	− 4.13	− 0.88
Estimated TM-score	0.28 ± 0.09	0.60 ± 0.14
Estimated RMSD	17.4 ± 2.6 Å	10.1 ± 4.6 Å

C-score is the confidence score of the predicted model. TM-score indicates the correct topology of the model. RMSD is the average distance between the atoms of superimposed proteins

omics studies, seven *T. palmi* proteins were shortlisted for possible interaction with GBNV glycoproteins (Table 2). GAXC02024257, GAXC02013569, GAXC02014620, GAXC02023408, GAXC02022757, GAXC02022757, and GAXC02015494 from the unannotated *T. palmi* transcriptome were considered to retrieve the gene sequences of adapter protein 2 (AP2), cathepsin B, cathepsin L, clathrin, C-type lectin, enolase, and vacuolar ATP synthase (vATPase) subunit E, respectively based on sequence similarity. The modelled proteins had acceptable Ramachandran plot and ProSA scores suggesting high quality of the models. The models of the proteins and ProSA and RAMPAGE analyses are shown in Supplementary Fig. 1. The amino acid residues in favored and allowed region for all these proteins ranged from 95.8 to 99.7%.

Predicted interaction of *T. palmi* and GBNV glycoproteins

The global scores of predicted protein complexes obtained from PatchDock and refined by FireDock are shown in Table 3. The Z-dock output of the site-specific docking of three best interactions with both G_N and G_C are shown in Supplementary Fig. 2. The representative interfacial interactions and Gibbs free energy values generated by PDBePISA are also shown (Supplementary Fig. 2). In case of G_N, the top interactions were observed with cathepsin L, C-type lectin, and enolase. The modelled G_N-cathepsin L

complex consisted of 12 interfacial hydrogen bonds and two salt bridges. The ΔG of the complex was −14.6 kcal/mol. A $\Delta G P$ value of 0.071 implies that the probability of obtaining a better complex than this was extremely small. G_N-C type lectin interaction featured 14 interfacial hydrogen bonds and one salt bridge. The ΔG of the complex was −11.8 kcal/mol with $\Delta G P$ value of 0.071. Finally, G_N-enolase complex had of six interfacial hydrogen bonds and one salt bridge with ΔG − 10.0 kcal/mol and $\Delta G P$ value 0.208.

In case of G_C, the top three interactions were recorded with C-type lectin, clathrin, and vATPase subunit E. G_C-C type lectin interaction was based on eight interfacial hydrogen bonds and three salt bridges. The ΔG of the complex was − 20.1 kcal/mol with $\Delta G P$ value 0.161. Eight interfacial hydrogen bonds and three salt bridges were involved in G_C-clathrin interaction. The ΔG of the complex was − 31.8 kcal/mol with $\Delta G P$ value of 0.023. Finally, the interaction between G_C and vATPase subunit E was supported by seven interfacial hydrogen bonds and two salt bridges. The ΔG and $\Delta G P$ value were recorded as − 31.8 kcal/mol and 0.023, respectively.

Discussion

GBNV-*T. palmi* is an important virus-vector combination with global impact on agricultural production. Effective management of this virus-vector complex may be possible by interrupting their close interrelationship, but limited genome information for *T. palmi* makes it challenging to determine the critical steps involved. Analysis of the available genome data for both *T. palmi* and GBNV will be pertinent to shed light on the molecular mechanisms of *T. palmi*-GBNV interaction. The present in silico study predicts that *T. palmi* proteins including cathepsin, C-type lectin, enolase, clathrin and vATPase subunit E are involved in direct interaction with GBNV glycoproteins to facilitate entry into thrips cells.

Table 2 *Thrips palmi* proteins predicted to interact with GBNV glycoproteins

Protein name	Accession no.	Protein function	References
Adaptor protein 2	GAXC02024257	Involved in clathrin mediated endocytosis (CME)	[1]
Cathepsin B	GAXC02013569	Glycoprotein maturation	[4, 11, 11]
Cathepsin L	GAXC02014620	Glycoprotein maturation	
Clathrin	GAXC02023408	Involved in CME	[1, 34]
C-type lectin	GAXC02022757	Glycoprotein binding	[21]
Enolase	GAXC02022757	Involved in glycolysis	[47, 3]
Vacuolar ATP synthase subunit E	GAXC02015494	Involved in CME	[3, 13]

Table 3 Global scores of protein complexes predicted from PatchDock and refined by FireDock

<i>Thrips palmi</i> protein	GBNV G _N		GBNV G _C	
	Global score	Z-score	Global score	Z-score
Adapter protein 2	1.91	− 1.73795	4.03	− 1.21349
Cathepsin B	− 3.48	− 1.19162	− 15.13	− 0.12936
Cathepsin L	− 28.27	1.321107	− 23.4	0.338578
Clathrin	− 18.2	0.300407	− 29.45	0.680905
C-type lectin	− 25.60	0.582189	− 47.06	1.677331
Enolase	− 20.41	0.524414	3.16	− 1.16426
Mitochondrial ATP synthase subunit α	− 18.32	0.31257	− 4.95	− 0.70538
Vacuolar ATP synthase subunit E	− 14.14	− 0.11112	− 26.53	0.515683

The sequences of several thrips genes that may be involved in interactions with GBNV envelope glycoproteins were retrieved from a transcriptome of *T. palmi* [25]. Watermelon bud necrosis virus (WBNV) is the closest known relative of GBNV. The predicted signal peptide and G_N-G_C cleavage site sequences (VYL-LN and PSSIA-LK, respectively) of WBNV [18] are similar with GBNV. The presence of signal peptide indicated the possible involvement of signal peptidase (SPs) and signal peptide peptidases (SPPs) in GBNV glycoprotein maturation. The role of SPs and SPPs in virus glycoprotein maturation is well documented [23]. In case of the bunyaviruses such as Hantaan virus and Bunyamwera virus, the involvement of SPs and SPPs in proteolytic maturation of glycoprotein has been experimentally proven [20]. Potential N-linked glycosylation and mucin-type O-glycosylation sites of GBNV glycoproteins have been identified in this study. Glycosylation is known to influence the folding and stability of glycoproteins. Several putative transmembrane domains were identified in GBNV G_{PP}. The transmembrane domains which take part in the membrane perturbing activity, are hydrophobic in nature. Several domains of low to medium hydrophobicity were observed in GBNV-G_C. These regions could be involved in the virion fusion in cytosol following many conformational changes. This is the first time such comprehensive sequence analyses of GBNV glycoproteins have been reported.

In protein–protein docking models, cathepsin L featured strong interaction with G_N. In case of (−) ssRNA viruses, cathepsin proteases are known to be involved in glycoprotein maturation and membrane fusion [4, 11]. Endosomal cathepsin proteases are known to be involved in the maturation of bunyavirus glycoproteins [11]. Recently, transcriptome-wide responses of adult *T. palmi* to infection by capsicum chlorosis virus (CaCV; genus *Tospovirus*) were determined. Cathepsin was found to be upregulated in CaCV exposed *T. palmi* [45]. However, the exact role of cathepsin L in tospovirus glycoprotein maturation remains to be studied. The in silico analyses indicated strong interaction of G_N with C-type lectin, a carbohydrate

binding protein. Bunyaviruses such as Rift Valley fever virus (RVFV) and Uukuniemi virus (UUKV) infect the animal cells with the help of the C-type lectin DC-SIGN. In vivo studies demonstrated that the dermal dendritic cells overexpressing DC-SIGN on their surface are more susceptible to RVFV, UUKV, Germiston virus, and Toscana virus (TOSV) [21]. DC-SIGN is specialized in capturing the N-glycan coat of high mannose residues present in the virus glycoprotein [36]. DC-SIGN is also involved in the internalization of bunyavirus particles as the cytosolic tail of DC-SIGN contains a dileucine (LL) motif which is critical for endocytosis. UUKV is unable to enter cells lacking the LL motif [21]. Apart from DC-SIGN, bunyaviruses also utilize L-SIGN, another C-type lectin [19]. This data and the results of the present analyses led us to hypothesize that GBNV may also interact with C-type lectin to enter thrips cells. On the other hand, G_C has the potential to form a strong complex with clathrin which plays an important role in the formation of coated vesicles during endocytosis. Several lines of evidence suggest that bunyaviruses first bind to a primary cellular receptor and then enter host cells by pH-dependent clathrin-mediated endocytosis (CME). Bunyaviruses such as Akabane virus, La Crosse virus, Oropuche virus, Tahyna virus, Crimean-Congo hemorrhagic fever virus have been shown to involve CME [1]. During this process, G_C appears to undergo several conformational changes due to acidic pH conditions in the endocytic vesicles. In the case of TSWV-infected *F. occidentalis*, clathrin and adaptor proteins are upregulated nine and 10-fold, respectively, implying the involvement of CME in viral internalization [34]. Under acidic conditions, G_C of TSWV is cleaved but not G_N. In the present analyses, hydrophobic domains were also observed in GBNV G_C which could be involved in virion fusion with vector cell membranes. Further, the interaction between GBNV G_C and clathrin with very low $\Delta G P$ value suggests that this interaction may be involved in the fusion process. In addition, GBNV G_C was predicted to form a complex with vATPase subunit E, a membrane-bound enzyme that catalyzes ATP-hydrolysis to transport the

solute across membranes and that is also involved in CME. In TSWV-infected *F. occidentalis*, vATPase subunit E is overexpressed, implying the involvement of CME [3]. vATPase is required for bunyavirus cell entry [13]. Our results predict strong interactions of GBNV-G_C with vATPase and clathrin implying that the entry of GBNV into thrips cells follows a CME. Furthermore, G_N and thrips enolase are predicted to show a strong interaction. Enolase is a metalloenzyme primarily involved in the glycolytic pathway. This prediction is supported by an earlier report of enolase from *F. occidentalis* directly interacting with purified TSWV particles. In TSWV-exposed *F. occidentalis*, enolase transcripts are upregulated [3]. Similar upregulation of enolase has been observed in the planthopper *Laodelphax striatellus* infected with rice stripe virus, which is related to bunyaviruses [47]. The present findings suggest that GBNV may affect the vector cell glycolysis cycle directly, but experimental evidence is required to support the in silico analyses.

In conclusion, based on our analyses we predict that C-type lectin likely is the first cellular receptor with which GBNV-G_N interacts to attach to thrips gut cells and then is internalized by endocytosis mediated by clathrin and vATPase. This data provides the first computational evidence of GBNV-*T. palmi* protein–protein interaction. Application of Yeast-2-hybrid analysis, bimolecular fluorescence complementation, pull-down assays, quantification of gene expression and omics studies will be required to provide the experimental evidence to support our bioinformatic predictions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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