Identification of whitefly (*Bemisia tabaci*) proteins interacting with *Tomato leaf curl Bangalore virus* coat protein gene using Y2H system

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Abstract

Tomato leaf curl Bangalore virus (ToLCBV) (Geminiviridae) causes the economically important tomato leaf curl virus disease (ToLCVD), and is transmitted by the whitefly, *Bemisia tabaci* (Gennadius) (Aleyrodidae: Hemiptera). Successful transmission of the virus by the insect requires safe translocation of the virus through different barriers inside the vector. During the translocation, the viral coat protein will interact with many whitefly proteins, while a few of them are beneficial to virus (e.g. heat shock proteins) but some may be harmful (e.g. whitefly immune proteins). We carried out the yeast two hybrid (Y2H) assays to identify the proteins of the *B.tabaci* Asia 1 genetic group interacting with ToLCBV coat protein (CP). The Y2H assay initially identified a total of 425 putative interacting whitefly proteins on a low stringent selection media, and they were reduced to 324 when the yeast colonies were grown on a high stringent media, and of which about 274 colonies produced single bands in colony PCR experiments while the remaining colonies produced multiple bands. Further, high selection pressure assays confirmed a total of 102 whitefly proteins interacting with ToLCBV CP and these included the heat shock proteins (HSPs) 70kDa, GroEL, nucleoproteins, vitellogenins, apolipophorins, 40s ribosomal proteins, sorbitol dehydrogenase, dipeptidyl peptidase, E3 ubiquitin, annexin, GTP cyclohydrolase, tropomyosin, salivary secreted proteins, succinate dehydrogenase, lachesins, enolase and

others. The identified proteins could be potential targets for novel whitefly control strategies such as using RNAi or insecticide target sites for developing future disease and whitefly control strategies.

Keywords: whitefly cDNA library, yeast two hybrid assay, Gemini virus coat protein

Introduction

Tomato (*Solanum lycopersicum*) is one of the most widely grown vegetable crops and popular due to its high nutritive value, taste and versatile use (Sidhu et al.2017). The crop is susceptible to more than 200 diseases, of which 40 are vector-borne (Martelli and Quacquarelli 1982; Lukyanemko 1991). Insect vector-borne plant viral diseases have a great importance worldwide. Among them, the tomato leaf curl virus disease (ToLCVD) caused by various strains of tomato leaf curl viruses (ToLCVs) (*Begomovirus*; *Geminiviridae*), is the most devastating and they are transmitted by the insect vector *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) (Kalloo 1991; Varma and Malathi 2003). Epidemics of ToLCVD associated with upsurge of *B. tabaci* on tomato has been frequently reported from Gujrat, India with up to 100% yield losses (Shelat 2014). Extensive economic losses caused by *B. tabaci* transmitted viral diseases have threatened global food security and poverty alleviation efforts with losses ranging from 20 to 100% (Cathrin and Ghanim 2014). The epidemiology of disease greatly depends on vectors and route of dissemination. The *B. tabaci* is very efficient in acquiring the virus and transmitting into potential new hosts (Rubinstein and Czosnek 1997). After acquisition of virus by the vector, the virus has to pass through many barriers inside the vector (eg., immune system) for successful transmission to healthy host plant (Sinisterra et al. 2005). During the process, virus interacts with various proteins for safe translocation and to overcome various types of insect immune reactions (Gray and Banerjee 1999; Power 2000). These interacting proteins may be beneficial to virus (e.g. heat shock proteins) and a few maybe harmful (e.g. immune proteins). A complex process thus exists

while multiplying and translocation of plant virus inside the insect vectors (Pakkianathan et al. 2015). Recent research also highlighted the role of symbiotic bacteria in the co-evolution of hosts and their parasites (Dheilly et al. 2015). Symbionts also known to play a crucial role in virus –vector interactions. During the process of symbiosis, many proteins including the HSPs and chaperons are secreted by endosymbionts inside the vector (van den Heuvel et al. 1994). They have dual roles; help in stress adaptation of the insect host and they are also used by viruses such a ToLCVs inside the vector to protect themselves from insect immune reactions (Oliver et al. 2003; Gutierrez et al. 2013). Plant virus interacts with HSPs produced by the endosymbiotic bacteria for safe circulative translocation in the vector leading to successful transmission to a new host and further virus spread (Gorovits et al. 2013). The GroEL is reported to protect the *Tomato yellow leaf curl virus* (TYLCV) from degradation during its passage through the *B. tabaci* haemolymph (Morin et al. 1999; Gotz et al. 2012). Likewise, there are many proteins inside the vector that interact with virus coat proteins which are less understood. The information on number of proteins that interact with *Tomato leaf curl Bangalore virus* (ToLCBV) inside the Indian indigenous whiteflies has not been investigated. There are many techniques for studying protein-protein interactions including co-immunoprecipitation, pull down assays, Split-ubiquitin System, yeast two hybrid (Y2H), bimolecular fluorescence complementation (BiFC), among others (Xing et al. 2016). Here we used the Y2H system to identify 102 whitefly proteins interacting with ToLCBV CP. Some of the proteins found were HSPs 70kDa, GroEL, nucleoproteins, vitellogenins, apolipophorins, 40s ribosomal proteins, sorbitol dehydrogenase, dipeptidyl peptidase, E3 ubiquitin, annexin, GTP cyclohydrolase, tropomyosin, salivary secreted proteins, succinate dehydrogenase, lachesins, enolase and others. These proteins can be targets for novel whitefly control strategies such as RNAi or insecticide target sites for developing future disease management approaches.

Material and Methods

Whitefly and virus cultures: A colony of the whitefly *B. tabaci,* Asia I species was originally collected from Coimbatore, India in the mid 2000s and maintained on egg plants with temperature 27± 3°C, 60% relative humidity and L12:D12 in the quarantine laboratories of the Natural Resources Institute (NRI), University of Greenwich, UK. The ToLCBV-infected tomato scions collected from the ICAR-Indian Institute of Horticultural Research fields (13°08'03.2"N 77°29'33.1"E), Hessarghatta Lake, Bengaluru, India were grafted on to healthy tomato seedling maintained in the quarantine glasshouse at NRI. Whiteflies total DNA was extracted from 10 female adults using the Chelex method (Walsh et al. 1991) with slight modifications (Ghosh et al. 2015).

Detection of virus

Disease symptoms were recorded by visual observation of the diseased plants (ToLCBV) and by the virus detection through PCR. Total DNA was extracted from the diseased tomato leaf samples using CTAB method (Lodhi et al. 1994; Maruthi et al. 2002). Molecular diagnosis of ToLCBV was carried using the end point PCR with newly designed primers (Forward 5' GTCAGCAATCTGCCAACGAC, Reverse 3' GTGTTGGATTGCCAGTCCCT) to amplify 475 bp size product of C1 gene. Primers were developed using complete genome sequences of ToLCVs available in the National Centre for Biotechnology Information (NCBI). Amplification of the gene was carried out in 15 µl volumes containing 1 µl DNA lysate (1:5 dilution) as template, 10 µM of each primer, 2 mM of dNTPs, 1.5 x DreamTaq Green buffer and 0.15 unit DreamTaq Green DNA polymerase (Thermo Scientific Ltd., UK). Amplifications consisted of 95°C for 1 min followed by 35 cycles of 94°C for 30s, annealing of 60°C for 15s and 72°C for 50s and final extension for 7 min at 72°C. PCR products were visualised on 1% agarose gels containing Sybrsafe staining solution (Invitrogen Biotechnology, USA).

Collection of healthy whiteflies for RNA extraction

Whitefly adults (100 females) from the core colony were released on healthy tomato plants for 48 h and were collected using aspirator, and immobilized with CO_2 before transferring them to liquid nitrogen in safe lock microfuge tubes.

Extraction of total RNA

The whitefly total RNA was extracted using protocols Clontech's (USA) Bioline kit (Bioline ISOLATE II RNA Mini Kit BIO-52072) + Zymo CC (Zymo RNA Clean & Concentrator™-5 # R1015) with slight modifications as below.

Whiteflies were removed from liquid nitrogen and homogenized using sterile plastic pestles in 350 µl of buffer RLY and 3.5 µl of 2-Mercaptoethanol, βME. Further steps were followed as indicated in the manufacture's protocol. The final elution was done twice, each with 50 µl of SDW and pooled. The 100 µl of purified RNA was cleaned and concentrated using Zymo CC kit, as per manufacturer's instructions except step 1 of the protocol, where no DNase was added. Final elution was done twice, each with 30 µl SDW and pooled. RNA quality and quantity was estimated using nanodrop ND2000 (Thermo Fisher Scientific Ltd., UK) and bioanalyser (Agilent Technologies Inc. USA).

The total RNA was precipitated into 2-3µl using a salt precipitation method by adding 0.1 volume of sodium acetate 3M, 5.2 pH, 2.5 volume of ethanol (100%) and incubated over night in -20^{o}C. After incubation, centrifugation at 11000g for 10 min was carried out and the supernatant was removed. To dissolve the salt, 500µl of ethanol (70%) was added and centrifuged at 4^{o}C for 10min at 11000g. The supernatant was removed and pellet was dried using a spin Vac at low temperature before adding 4µl of RNAse-free water. The quality and quantity of RNA was tested using 1 µl in a nanodrop (0.10–2.0 µg of total RNA was needed) in the mixture and remaining quantity was used for preparing cDNA library.

Preparation of whitefly cDNA library

Clontech's (USA) "Make your own plate & mate" Library system user manual was followed to develop the whitefly cDNA library from the extracted total RNAs.Total 4µl reaction mixture consisted of 2µl with 1.55ng of total whitefly RNA, 1 µl each of CDS III enzyme and sterile water, incubated at 72^{o}C for 2min. Further steps of library preparation were followed as per the manufacturer's instructions. Final incubation at 37^{o}C at 20min was done to deactivate RNase H which was added as a part of the protocol.

After the first strand cDNA synthesis from above, the amplification of cDNA was carried out using Long Distance PCR (LD-PCR) as per the manufacture's protocol (Clontech, USA). The final PCR product was visualised on a 1.2% agarose gel and purification of DNA size >200bp was carried out using QIAquick PCR cleaning kit (Qiagen, USA). Quality of cDNA was checked using the nandodrop. About 4.24 µg of double stranded cDNA in 20 µl was required for constructing the yeast two hybrid cDNA library.

Cloning whitefly cDNA library for Y2H assays and transformation into yeast

Yeastmaker Yeast Transformation System 2 User Manual (Clontech, USA) was followed for co-transformation of vector and cDNA. The preparation of yeast competent cells, *Saccharomyces cerevisiae* Y187 (strain) was carried out by streaking of YPDA agar plate with *S.cerevisiae* Y187 strain from a frozen yeast stock and incubated the plate upside down at 30° C until colonies appeared (~3 days). The remaining procedure was carried as per the manufacture's protocol.

The transformation of Y187 competent cells was carried out as per the manual (Clontech, USA). After transformation, 15 ml solution was used to spread about 100 petri dishes (150mm diameter) containing the SD/-Leu and incubated at 30^{o}C for 3-5 days. Plates were then chilled at 4°C for 3–4 hr followed by addition of 5 ml of freezing medium and 5-6 sterile

glass beads to detach the colonies from each plate. At the end, all the colonies were collected in a single sterile flask for developing the composite whitefly cDNA library in yeast cells. The prepared library was aliquoted into several 1 ml aliquots for short-term use and a few 50 ml aliquots for long-term storage at –80°C. Only 1ml aliquot was used for screening according to the Clontech's (USA) Matchmaker Gold Yeast Two-Hybrid System.

Preparing ToLCBV CP bait for the Y2H assay

Full length ToLCBV CP gene from base position 301 to 1071 on the virus genome was amplified using infusion Smart technology as per Clontech's protocol (USA). Infusion primers were designed by incorporating 15bp PGBKT7 vector sequences (5'- GATCCGTCGACCTGC; 3'-GTACCTCCGGCTTAA) to the primers (ToLCBV F-CATGGAGGCCGAATTATGTCCAAGCGTCCGGCAG; ToLCBV R-CAGGTCGACGGATCTCAATTGGTTACAGAATCGTAGAAG).

PGBKT7-DNA BD vector was digested with *BamH1* HF and *EcoRI* HF enzymes (NEB, UK) with cut smart buffer at 37^{o}C for 30 min followed by deactivation of enzymes at 65^{o}C for 20min. The resultant product was visualized on 1% agarose gel with 6X Sybrsafe tracking dye. The Gel bands were cut and extracted using the Nucleospin gel extraction kit (ThermoFisher Scientific,UK) as per the manufacture's protocol and total DNA quantity was analysed using the nanodrop to confirm the requirement of 50-100 ng linearized vector for cloning.

Cloning was carried out by adding the insert (amplified CP products) to the linearized vector with a ratio of 2:1. A total of 10 µl of reaction mixture containing 4µl of 124ng of CP DNA, 4µl of 56ng linearised vector and 2 µl 5X-In-Fusion HD enzymes premix (Clontech, USA) was incubated at 50^{o}C for 15 min for ligation and then placed on ice.

E. coli stellar competent cells with transformation efficiency ≥ 1X 108cfu/µg from Clontech's (USA) infusion HD cloning kit was followed. The resultant mixture was spread on a separate LB plate containing the antibiotic, kanamycin sulphate (KAN 50µg/ml) and incubated overnight at 37°C. Colony PCR was performed as above to confirm the cloning of ToLCBV CP into the PGBKT7-DNA BD vector. The positive colonies were inoculated in 8ml LB broth with 8 µl of KAN 50 at 37ºC with shaking overnight. Next day 700 µl culture was aliquoted into 1.5ml centrifuge tube and 300 µl of 50-75% glycerol added and mixed properly and stored in -80ºC for future use. The remaining culture was used for plasmid isolation.

The culture was centrifuged at high speed (11000g) for 5min; supernatant was discarded. Thermo scientific's Mini plasmid isolation kit was used to isolate the plasmid and quantity of DNA was estimated using the nanodrop. The samples were sent for sequencing by mixing with 5 µl of 5µM of forward and reverse primer separately for confirmation of cloning.

Yeast transformation of coat protein gene

Streaking of YPDA agar plate with *S. cerevisiae* Y2H gold strain from a frozen yeast stock and incubated the plate upside down at 30°C until colonies appeared (~3 days). All the other procedures followed were similar to preparation of Y187 competent cells in cDNA library preparation above and as per manufacturer's instructions. The transformation was also similar to cDNA library preparation except a small-scale transformation was carried out. One plate of SD/-trp (CSM, For medium, UK) was used to spread the transformed yeast cells and incubated at 30ºC for 3-5 days. Transformation was confirmed through aforementioned colony PCR. Single positive fresh colony (2-3mm) containing the bait strain was inoculated in SD/-trp broth medium and incubated at 30ºC shaking for 16-20hr until 0.8 OD600 was reached. The culture was then centrifuged at 1000*g* for 5 min and supernatant was discarded.

196 The pellet thus obtained was resuspended in SD/-trp (4-5ml) to obtain cell density of >1 x
197 10^8cell/ml by counting cells using a haemocytometer.

198 **Mating of Asia I *B. tabaci*c DNA library and the bait protein ToLCBV CP in the**
199 **Matchmaker Gold Yeast two hybrid system**

200 One ml of the cDNA library Y187 cells stored in -80^{o}C was thawed at room temperature and
201 mixed with 4-5ml of bait cells of Y2H Gold strain obtained in a one liter conical flask.
202 Further 45ml of 2xYPDA medium (with kanamycin 50µg/ml) was added into the conical
203 flask containing library and bait. The mixture was incubated at 30^{o}C for 24 hrs with slow
204 shaking (30-50 rpm). After 20 hrs, a drop of the culture was tested for zygotes (3 lobbed
205 structures) under phase contrast microscope. After confirmation, the suspension was
206 centrifuged at 1000*g* for 10 min to get pellets. Meanwhile, 1L conical flask was rinsed twice
207 with 50ml of 0.5X YPDA (with kanamycin 50µg/ml) and combined both rinses and used the
208 rinse to resuspend the pelleted cells. The cells were centrifuged to get pellets and resuspended
209 in 10ml of 0.5X YPDA/KAN liquid medium. About 200µl of suspension was spread on each
210 150mm petri plate containing triple drop out media (TDO) (SD/-His/-Leu/-Trp) (CSM,
211 Formedium, UK). The culture was spread on 55 plates which were incubated at 30^{o}C for 3-5
212 days for colony growth. Each colony was subsequently transferred onto higher stringency
213 medium (QDO) (SD/-Ade/-His/-Trp/-Leu) (CSM, Formedium, UK) with X-α-Gal (20mg/ml
214 of media) and aureobasidin antibiotic (150ng/ml of media). Then all QDO/X/A positive
215 interactions were further analysed to identify duplicates and to verify genuine interactions.
216 The blue colonies on QDO plates were marked and subjected to colony PCR by using 1 part
217 of each 10µm of T7 (forward) and 3AD (reverse) primers with the remaining reaction as
218 above to check the single bands on 1% gel. The colonies that produced single bands were
219 sent forsequencing after purifying their respective PCR products as per Thermo Scientific's
220 PCR purification kit. The remaining colonies that produced more than one band were re-

streaked on QDO/X/A for separating them and incubated for 3-5 days. The colony PCR was repeated as above.

Results

Whitefly and virus detection, and confirming the cloning steps

About 870bp product of the whitefly mtCoI DNA was amplified, and 475 bp product of the ToLCBV CP gene were amplified for diagnostic purposes (Acc. No. MN752118). Likewise, the complete CP gene (771 bp) of ToLCBV was also amplified for constructing the bait (Acc. No. MK120481), and all sequences have been submitted to NCBI gene bank. The linearised pGBKT-7 vector was digested (7.3kb) and the amplified ToLCBV CP are shown in Figure 1a and 1b. The successful transformation of ToLCBV CP in *E. coli* was confirmed by amplifying colony PCR amplification of ToLCBV CP (771 bp) (Figure 2a). Presence of transformed ToLCBV CP into Y2H gold was confirmed by amplification of CP gene (Figure 2b).

Protein-protein interactions

The Y2H assay was carried out to identify the number and type of whitefly proteins interacting with ToLCBV CP. Initial results revealed 425 interactions on the low stringent triple dropout media (TDO) (media lack of histidine, leucine and tryptophan), followed by 324 interactions on high stringent media called quadruple drop out media (QDO), which lack aminoacids such as adenine, histidine, tryptophan and leucine. Out of the 324 colonies, about 274 produced single bands in colony PCR. Upon further selection pressure on the highest stringent media (QDO) and through re-streaking of remaining colonies, a total of 102 positive interactions were found to interact with ToLCBV CP at all stringency levels (Table 1). Some of these proteins foundto be interacting with ToLCBV CP were HSPs 70kDa, GroEL, nucleoproteins, vitellogenins, apolipophorins, 40s ribosomal proteins, sorbitol

dehydrogenase, dipeptidyl peptidase, E3 ubiquitin, annexin, GTP cyclohydrolase, tropomyosin, salivary secreted protein, succinate dehydrogenase, lachesins, and enolase among others.

Among the interactions, the maximum interactions were by the vitellogenin-A1-like mRNA (31 clones; 30.39%), acyl-CoA delta-9 desaturase (npve gene) (23 clones), *Candidatus Portiera aleyrodidarum* strain China 1 chromosome (16 clones), nucleoprotein TPR-like mRNA (10 clones), Asia I vitellogenin mRNA (7 clones), apolipophorins (7 clones), 40S ribosomal protein S20, mRNA (6 clones), sorbitol dehydrogenase (4 clones), venom dipeptidyl peptidase 4, E3 ubiquitin-protein ligase HUWE1, high affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8B, elongation factor 1-alpha, heat shock 70 kDa protein (3 clones each), lachesin, enolase, succinate dehydrogenase (2 clones each).

Discussion

The specificity of a plant virus transmission by any insect vector involves a complex interaction (Andret-Link and Fuchs 2005). The association of plant virus and vector facilitates the virus to survive and spread from plant to plant. To aid the process within the vector, viruses interact with vector's protein (Whitefield et al. 2015). We identified about 102 *B. tabaci* proteins interacting with ToLCBV CP. The functions of these proteins have been identified based on available literature and found many of them are involved in insect immunity and a few known to interact with the virus. Proteins involved in insect immunity are lachesin, which helps in morphogenesis of whitefly tracheal system; acyl-CoA delta-9 desaturase (npve gene) reported to have heat adaptation by regulating lipid desaturase (Llimargas et al. 2004; Ma et al. 2015). Likewise, another protein found was apolipophorins, which play an important role in lipid transport and insect innate immunity and multicellular encapsulation (Whitten et al. 2004; Zdybicka-Barabas and Cytryńska 2013). Whereas the 12

kDa FK506-binding protein is a receptor for the immune-suppressant drug which was also found to interact with coat protein of ToLCV (Aghdasi et al. 2001). Some more proteins associated with immune system are E3 ubiquitin-protein ligase HUWE1, which helps in degradation of foreign proteins. For example, ubiquitin-proteasome helps the whitefly to counteract the negative influence from TYLCV through degrading the virus directly or by activating the immune response (Gorovits and Czosnek 2017). Similarly, ATP-dependent RNA helicase p62 plays a role in innate immunity in mosquito, specifically restricts bunya virus infection, including *Rift Valley fever virus* (RVFV) or *La Crosse virus* (LACV) (Moy et al. 2014). In the same way, muscle M-line assembly protein unc-89 involved in immune response and F-BAR domain only protein 2 in endocytosis, exocytosis and cell motility (Wilson et al. 2012). Another protein 'coatomer subunit delta', a vesicle transporter reported to play role in cellular processes beyond golgi–endoplasmic reticulum retrograde protein trafficking (Isoe et al. 2011). Whereas, RING finger protein 10 and twitchin in autophagy and immunity, respectively (Xu et al. 2014).

In contrast, some proteins involved directly or indirectly in helping the movement of virusesthrough the body of insect vector or counteract the negative effects of the insect immunity were also identified. The protein 'elongation factor 1-alpha' not involved in regulation of apoptosis or cytoskeleton formation but interacts with the *West Nile Virus 3′ SL* and *Tobacco mosaic virus* and facilitates the synthesis of viral minus-strand (Zeenko et al. 2002; Li et al. 2013). 'TPR-like' nucleoproteinsare directly involved in the encapsidation of the virus genome for the purposes of RNA transcription, replication and packaging (Portela and Digard 2002). Likewise, 'PDZ and LIM Zasp-like domain protein' found in this studyis expressed in the midgut of insects for muscle strengthening but it is also a member of PDZ and LIM proteins involved in breast cancer progression in humans (Liu et al. 2015). 'Vitellogenin-A1-like' was an important protein found interacting with the CP, which is

essential in embryogenesis i.e. development of oocytes and embryo (Atella et al. 2005; Jorgensen et al. 2009). In this study, about 31 clones were found to be interactive with CP, probably due to vertical transmission of ToLCBV to next generation through eggs. A '40S ribosomal protein S20' also interacted with the CP of ToLCV, which is involved in subcellular localization, protein activity regulation, protein with binding function or cofactor requirement and transcription (Zhou et al. 2015; Lost and Jain 2019). Similar interaction was reported in *Arabidopsis thaliana* with *Turnip mosaic virus* infection (Yang 2007). Some disease manipulative proteins such as 'zinc finger X-linked protein ZXDA-like', a DNA binding protein, which are able to target specifically and manipulate disease-causing genes (Cassandri et al. 2017) was also found. Some proteins are reported to be involved in acquisition and movement of viruses in the vector (Wezel et al. 2003; Garcia and Damonte 2007). 'Tropomyosin-1'has been involved in the acquisition and movement of TYLCV in the insect body (Shalev et al. 2016) was also found to interact with ToLCBV in the current study.

In order to transmit a virus to healthy plant, some salivary secreted proteins in insect vector facilitate the virus (Dietzgen et al. 2016). In the present study, probable 'salivary secreted peptide' was found which may involve in recognition and transmission of begomoviruses. Earlier, proteins such as heat shock 70 kDa protein cognate 3, a endosymbiont derived protein known to interact with TYLCV and the bipartite *Squash leaf curl virus* (Ghanim and Medina 2007;Gorovits and Czosnek 2017; Kumar 2019) was also found in this study. Likewise, 'enolase', is found to be identical to the heat shock protein 'HSP48'; expression of an enolase protein was up-regulated in the midgut of *Aedes aegypti* infected with chikungunya or dengue viruses but its role with ToLCBV needs confirmation. Another protein 'HIRA-like' found in *Wolbachia*-infected insects and expressing cytoplasm incompatibility with non-infected individuals. Similar to this, a 'nucleolin' which was found to co -occur with HSP 70, probably play similar role like HSP

70. CPA GroEL BT-H 1 GroEL gene, an endosymbiont protein helps in protein folding also observed to be interacted with CP. Similarly, *Candidatus Portiera aleyrodidarum* chromosome 1 which is involved in unfolding of stressed protein in the vector also known to interact with ToLCBV CP in 16 clones. Previously, a 63-kDa GroEL protein produced by the *Hamiltonella* and *Aresenophonus* in *B. tabaci* has been reported to interact with CP of TYLCV and Cotton leaf curl virus respectively, during its passage through the haemolymph (Morin et al. 1999; Gottlieb et al. 2010; Rana et al. 2012). However, the new proteins found in this study need to be explored further to know the genuine interactions.

Future line of work

Confirming role of the identified proteins through one-to-one interactions and silencing such genes by RNAi or by other methods would be the first line of future work to be carried out. The proteins can also be target sites for novel virus blocking strategies in future studies. Disrupting virus movement in the vector can be a novel approach for preventing virus spread to healthy plants under field conditions. All this work can greatly contribute to developing methods for efficient management of whiteflies and viruses they transmit.

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Compliance with ethical standards

No potential conflict of interests to be disclosed.

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