

1                   **Deciphering cassava brown streak virus infection in cassava through VPg**  
2   **mediated host protein interactions**

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8 **Abstract :**

9 Cassava brown streak disease (CBSD) poses a major threat to cassava production in Africa. Identifying cassava  
10 proteins that interact with cassava brown streak virus (CBSV), the major causal virus, can help elucidate the  
11 mechanisms of infection and resistance. Here we constructed a cassava cDNA library and screened for proteins  
12 interacting with CBSV viral genome-linked protein (VPg) using yeast two-hybrid assays, identifying 36  
13 interactors. Four candidates were validated in *Nicotiana benthamiana* via bimolecular fluorescence  
14 complementation. Functional categories included chloroplast proteins, ribosomal components, chaperones,  
15 metabolic enzymes, and defence-related proteins. In prior RNA-seq datasets from CBSV or UCBSV-inoculated  
16 cassava, comprising the susceptible variety Albert and the resistant variety Namikonga, 16 VPg-interacting genes  
17 were identified among the differentially expressed genes ( $|\log_2FC| > 1$ ), with 2 detected in Albert and 15 in  
18 Namikonga. These results indicate that VPg-interacting host proteins are involved in CBSV infection dynamics  
19 and also explaining the differential responses of resistant and susceptible cassava varieties, thus offering potential  
20 new molecular targets for CBSD management.

21  
22 **Keywords:** *Cassava brown steak virus*, VPg, Yeast two hybrid, Protein-protein interaction, cDNA library,  
23 chloroplast.

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## 1 Introduction

2 Cassava (*Manihot esculenta* Crantz) grown mainly for its starchy tuberous roots is consumed by approximately  
3 800 million people worldwide (1,2). It is a food security crop in many countries of Sub-Saharan Africa (SSA)  
4 and is gaining importance as an industrial crop especially for its use in the production of starch. However, the  
5 emergence of two viral diseases, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) has  
6 severely affected cassava production and food security in SSA (3). With the recent spread of CMD to East Asia  
7 and the expected spread of CBSD to West Africa, they have been considered the greatest threat to cassava  
8 production globally (4,5). More than half of the cassava plants grown in Africa are affected by CMD and CBSD  
9 that results in annual production losses of more than US\$ 3 billion (6,7). CBSD can reduce production up to 70%  
10 of the total root yield in susceptible varieties (8). Analysis of whole genomes of several viruses isolated from  
11 CBSD-infected plants revealed the presence of two distinct virus species: Cassava brown streak virus (CBSV)  
12 and Ugandan cassava brown streak virus (UCBSV) (genus *Ipomovirus*, family *Potyviridae*) (9–11), which are  
13 collectively called cassava brown streak viruses (CBSVs). CBSV is transmitted by the insect vector, whitefly,  
14 *Bemisia tabaci* (12), although field transmission is predominantly by infected stem cuttings.

15 Plant host factors play a crucial role in the infection process of CBSV, as is the case with many plant viruses.  
16 Successful infection depends on complex interactions between viral and host factors that facilitate viral genome  
17 replication, as well as cell-to-cell and long-distance movement. Viruses reshape the intracellular environment by  
18 interacting with pro-viral host factors, co-opting essential cellular processes, and interfering with host antiviral  
19 defences. On the contrary, interactions with antiviral host factors determine the effectiveness of plant defence  
20 mechanisms, such as the hypersensitive response and post-transcriptional gene silencing, and influence the virus's  
21 ability to overcome these defences (13,14). Some of these interactions may also contribute to disease symptom  
22 development, either directly or through collateral effects unrelated to virus replication (15). Due to the constraints  
23 of the viral genome size, and associated limited coding capacity, the viral proteins are multifunctional and have  
24 evolved to target various host factors (16). The viral protein VPg, is essential for infectivity. It binds to many plant  
25 proteins as it is a hub controlling processes involved in the multiplication and spread of viruses (17,18).

26 Identifying these viral-host protein interactions is key to understanding CBSV resistance mechanisms. High-  
27 throughput protein–protein interaction studies, such as yeast two-hybrid (Y2H) screens, are effective for  
28 elucidating plant-virus interactomes and the molecular pathways targeted by viruses (19–21). Y2H has been used  
29 extensively to identify ‘bait proteins’ interacting with ‘prey proteins’ encoded by a cDNA library (22). Many plant  
30 proteins interacting with viral proteins have been identified through Y2H, thus helping understand the molecular  
31 basis of viral infection and the host defence mechanisms (20). RNA sequencing (RNA-seq) has emerged as a  
32 powerful tool for uncovering host gene expression changes during plant-virus interactions, enabling the  
33 identification of key genes involved in resistance or susceptibility (23,24). In cassava, RNA-seq has been  
34 instrumental in comparing resistant and susceptible genotypes upon CBSV infection, revealing genotype-specific  
35 defense responses and regulatory pathways (25–27). These studies demonstrated that transcriptomic profiling  
36 could identify differentially expressed genes (DEGs), which may play critical roles in infection and defense.

37 In this study, we used the CBSV VPg protein as bait to identify interacting host proteins from a newly synthesized  
38 cassava cDNA library. We further validated the *in-planta* interactions of four selected candidates in *Nicotiana*  
39 *benthamiana* using a bimolecular fluorescence complementation (BiFC) assay for validating the identified

40 interactors. To assess the relevance of these interactions during infection, we examined the expression patterns of  
41 VPg-interacting genes among the DEGs identified in previous studies of resistant (Namikonga) and susceptible  
42 (Albert) cassava varieties following viral infection (25,27). The VPg-interacting host proteins identified in this  
43 work provide new insights into the CBSV infection process and represent potential targets for enhancing cassava  
44 resistance to the virus.

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## 1 **Materials and methods**

### 2 **Plant materials and virus source**

3 Cassava variety Albert plants were grown in the NRI glasshouse at  $28 \pm 5$  °C with 50-60 % relative humidity. The  
4 CBSV- [TZ: Nal:07] strain (28) was graft inoculated and maintained in cassava was used in this study.

### 5 **RNA isolation and Y2H cDNA library construction**

6 Total RNA was extracted from cassava leaves using a combined CTAB-based DNA extraction method followed  
7 by purification with the RNeasy Plant Mini Kit (Qiagen, Germany) as described by (29). Samples were treated  
8 with DNase I, and mRNA was purified using the Oligotex mRNA Mini Kit (Qiagen, Germany).

9 A cassava Y2H cDNA library was constructed using the Make Your Own *Mate & Plate*<sup>™</sup> Library System  
10 (Clontech, USA). First-strand cDNA was synthesized using SMART technology, followed by 20-cycle LD-PCR  
11 amplification. Double-stranded cDNA (>200 bp) was size-selected, co-transformed with SmaI-linearized  
12 pGADT7-Rec into *Saccharomyces cerevisiae* strain Y187, and plated on SD/–Leu medium. Transformed colonies  
13 were pooled to generate the library, and library quality parameters, including cell density, were assessed according  
14 to the manufacturer’s instructions.

### 15 **Y2H assays**

16 All Y2H assays were performed using the Matchmaker Gold Yeast Two-Hybrid system (Clontech, USA). The  
17 bait was expressed from pGBKT7 as a fusion with the GAL4 DNA-binding domain, while prey proteins were  
18 expressed from the cassava cDNA library in pGADT7 fused to the GAL4 activation domain. *Saccharomyces*  
19 *cerevisiae* strains Y2HGold (bait) and Y187 (prey) were used. For screening, bait and prey strains were mated  
20 and plated on TDO (Triple dropout medium: SD/–His/–Leu/–Trp) for initial selection, followed by QDO/X  
21 (Quadruple dropout medium: SD/–Ade/–His/–Leu/–Trp with X- $\alpha$ -Gal) for high-stringency selection. Positive  
22 colonies were verified by colony PCR and sequencing.

23 For library validation, the cassava  $\beta$ -tubulin gene (Manes.09G100400.1) was cloned into pGBKT7 using the In-  
24 Fusion HD cloning kit (Clontech) and transformed into *E. coli* JM109, then into yeast strain Y2HGold following  
25 the manufacturer’s protocols.

### 26 **Screening for VPg-interacting proteins**

27 For the Y2H assay to identify the VPg interacting cassava proteins, the VPg bait vector was synthesised by  
28 amplifying CBSV VPg gene using gene-specific primers (Table SI 1) and cloned into the pGBKT7 vector. The  
29 pGADT7-BD-VPg was transformed into yeast using the Yeastmaker<sup>™</sup> Yeast Transformation System 2 kit  
30 (Takara Bio USA, Inc, California, USA). Auto-activation of the bait in yeast was assessed by monitoring growth  
31 after 3–5 days at 30 °C on SD/–Trp, SD/–Trp/X- $\alpha$ -Gal, and SD/–Trp/X- $\alpha$ -Gal/AbA plates, while toxicity was  
32 evaluated by comparing the growth of yeast transformed with pGBKT7-BD-VPg to those transformed with the  
33 empty pGBKT7 vector on SD/–Trp plates.

34 For Y2H screening, Y2HGold cells expressing the VPg bait were mated with the cassava cDNA library in strain  
35 Y187. Serial dilutions of the mated culture were plated on SD/–Trp, SD/–Leu, and SD/–Leu/–Trp (DDO) media  
36 to estimate the number of clones screened. Positive interactions were selected on QDO/X (SD/–Ade/–His/–Leu/–

37 Trp supplemented with X- $\alpha$ -Gal). Colonies growing on QDO/X plates were analyzed by colony PCR, and inserts  
38 were sequenced. Clones yielding multiple amplicons were re-streaked on QDO/X plates until single inserts were  
39 obtained. Sequence data were used to identify corresponding genes and proteins using Phytozome and NCBI  
40 databases. Redundant clones were removed, and one representative clone per gene was selected for further  
41 analysis. Subcellular localization was predicted using MultiLoc2 (30), and gene ontology analysis was performed  
42 using the PANTHER database.

#### 43 **Plasmid rescue and reconfirmation of the interaction by one-to-one Y2H assay**

44 To validate interactions by one-to-one Y2H assays, pGADT7-AD-cDNA plasmids were rescued from positive  
45 library clones and transformed into *E. coli* DH5 $\alpha$ . Prey plasmids from ampicillin-resistant colonies, confirmed by  
46 colony PCR for correct insert size, were retransformed into yeast. Individual prey-expressing Y187 strains were  
47 then mated with VPg bait-expressing Y2HGold cells, and interactions were confirmed by growth on TDO plates  
48 followed by high-stringency selection on QDO/X plates.

#### 49 **Bimolecular fluorescence complementation (BiFC)**

50 To confirm the *in-planta* interaction between cassava genes and the VPg protein, a BiFC analysis was performed  
51 in *Nicotiana benthamiana* using a subset of four genes selected based on their presumed importance in CBSV  
52 infection process. The four cassava genes were: Bcl-2 (B-cell lymphoma 2)-associated athano gene 1 (BAG 1,  
53 XP\_021619128.1), chaperone protein dnaJ A6 (DjA6, XP\_021615889.1), Probable inactive receptor-like protein  
54 kinase At3g56050 (PIRPK, XP\_021625915.1), and Scarecrow-like protein 8 (SCL8, XP\_021592369.1). The  
55 BiFC vectors pSPYNE and pSPYCE (N- and C-terminal YFP fragments) were obtained from Prof. Dr. Jörg Kudla  
56 (University of Münster, Germany). BiFC constructs were generated by cloning cassava genes into pSPYCE and  
57 VPg into pSPYNE using the In-Fusion HD cloning kit (Clontech, USA). Genes were PCR-amplified with primers  
58 (Table SI 2), purified, and inserted into BamHI-linearized pSPYNE or KpnI-linearized pSPYCE vectors.  
59 Agrobacterium clones were prepared, and working suspensions of paired constructs (Table SI 3) were used for  
60 agroinfiltration into *Nicotiana benthamiana* leaves following (31). Leaf segments (5  $\times$  5 mm) were collected three  
61 days post-infiltration and imaged for fluorescence using a Zeiss LSM 880 confocal microscope at 20 $\times$   
62 magnification. YFP reconstitution was monitored using an argon ion laser at 488 nm, with excitation at 514 nm  
63 and emission at 520–540 nm.

#### 64 **Expression analysis of VPg-interacting cassava genes**

65 To explore the relationship between VPg-interacting cassava proteins and CBSVs resistance, gene expression  
66 profiles were analysed in previously published RNA-seq datasets. Differential gene expression data listed in the  
67 studies (Amuge et al., 2017; Maruthi et al., 2014) which analysed CBSV responses in two cassava genotypes with  
68 contrasting resistance phenotypes: Albert (CBSV-susceptible) and Namikonga (CBSV-resistant, also known as  
69 Kaleso), were retrieved (Supplementary materials excel file 1 & 2) and analysed. Log<sub>2</sub> fold-change (log<sub>2</sub>FC)  
70 values comparing CBSV-infected and mock-inoculated plants were extracted for all genes encoding VPg-  
71 interacting proteins identified in this study. In these studies, expression data encompassed multiple infection  
72 stages, which was further grouped here as early (2, 5, and 8 days after grafting [DAG]) and late (45 DAG and 1  
73 year after grafting [YAG]) time points. DEGs were selected based on a  $|\log_2FC| > 1$  threshold. The number and

74 direction of DEGs were determined separately for each genotype and time point, and results were summarized to  
75 compare transcriptional responses between resistant and susceptible varieties.

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## 1 **Results**

### 2 **Cassava cDNA library preparation and VPg bait quality assessment**

3 To enable Y2H screening for CBSV-VPg interactors, a cassava cDNA library was constructed and quality  
4 assessed. Electrophoresis showed a uniform cDNA smear from 0.1–3 kb (Fig SI 1). The library comprised  $\sim 2.22$   
5  $\times 10^6$  independent clones, with a transformation efficiency of  $7.4 \times 10^5/\mu\text{g}$  pGADT7-rec and a cell density of  $4.38$   
6  $\times 10^8$  cells/ml. Insert sizes ranged from 300 to 1800 bp (average insert size of 765 bp; Fig SI 2), indicating  
7 sufficient quality for subsequent Y2H screening. To validate the Y2H cDNA library,  $\beta$ -tubulin was used as bait  
8 in a Y2H assay yielding more than 500 colonies on high-stringency QDO/X plates. Sequencing of 50 clones  
9 identified 42 annotated cassava genes, five unannotated loci, and three without matches (Table SI 4; Fig. SI 3),  
10 including known  $\beta$ -tubulin interactors (Table SI 5; Fig. SI 4). These results confirmed the high quality of library.  
11 CBSV VPg, used as bait, showed no toxicity in yeast and no autonomous activation, as indicated by normal growth  
12 on control plates (Fig 1a) and the absence of colonies on SDO/X/A plates (Fig 1b).

### 13 **Y2H screening of CBSV VPg interactors**

14 The mated culture of the bait strain (pGBKT7-BD-VPg) with the cassava cDNA library yielded 136 colonies on  
15 49 TDO plates (Fig 1c), of which 96 developed blue colours on QDO/X medium plates, indicating positive  
16 interactions (Fig 1d). Colony PCR analysis showed single amplicons in 82 colonies, multiple amplicons in 10  
17 colonies, and no amplification in four colonies. Clones with multiple amplicons were repeatedly streaked on  
18 selection media until a single amplicon was obtained. Sequencing of these clones identified 54 potential cassava  
19 interacting proteins. Subsequent one-to-one Y2H assays using rescued prey plasmids and the VPg bait resulted in  
20 42 blue colonies on QDO/X plates (Fig 1e), from which 36 proteins were identified by NCBI BLAST search  
21 (Table 1).

### 22 **Gene ontology of VPg-interacting cassava proteins**

23 Gene ontology classification of the interactors revealed molecular function (Fig 2a) and catalytic activity as the  
24 predominant class, followed by binding activity. In terms of biological processes (Fig 2b), the main categories  
25 were cellular and metabolic processes. For cellular components (Fig 2c), most proteins were associated with cell  
26 parts and organelles, while nucleic acid binding was the major protein class identified (Fig 2d).

### 27 ***In-planta* interactions of cassava proteins through BiFC**

28 The *in-planta* interactions of four cassava proteins; BAG1, DjA6, SCL8, and PIRPK, selected based on their  
29 presumed roles in the CBSV infection process, were validated by BiFC analysis in *Nicotiana benthamiana*.  
30 Fluorescence signals were detected for all four cassava proteins (Fig 3a), confirming their *in-planta* interactions  
31 with VPg in *N. benthamiana*. The fluorescence signals were observed at both the cell periphery, cytoplasm and  
32 in the nucleus. Strong fluorescence signals indicating the BAG1–VPg interaction were predominantly observed  
33 in the cytoplasm, although the speckled pattern may be indicating fluorescence originating from plastids. Weak  
34 fluorescence signals corresponding to the DjA6–VPg interaction were detected in the cytoplasm. Fluorescence  
35 signals for the SCL8–VPg interaction were observed in both the cytoplasm and the nucleus, with nuclear signals  
36 suggesting localization at the nuclear membrane. Fluorescence signals for the PIRPK–VPg interaction were also  
37 observed in the periphery which might indicate cytoplasm or membrane-based interaction.

### 38 **Expression analysis of the identified VPg-interacting cassava proteins**

39 To investigate the relationship between the VPg-interacting cassava proteins and the observed differential  
40 resistance to CBSVs, we examined their gene expression profiles in resistant Namikonga and susceptible Albert  
41 cassava variety. Log<sub>2</sub> fold-change values under both control and CBSVs -infected conditions were extracted from  
42 two previously conducted studies (25,27). Across both studies, 22 of the 36 VPg-interacting genes identified were  
43 found to be differentially expressed between virus infected and uninfected plants when considering both the Albert  
44 and Namikonga varieties combined. After applying a  $|\log_2 \text{fold change}| > 1$  threshold, this number was reduced  
45 to 16 genes (Table SI 6); of these, 2 DEGs were identified in Albert and 15 in Namikonga. We further classified  
46 these DEGs by infection stage into early time points (2, 5, and 8 DAG) and late time points (45 and 54 DAG, and  
47 1 YAG). In the Albert variety, no early stage DEGs were found but at the late stage (45 DAG and 1 YAG,  
48 respectively) BAG family molecular chaperone regulator 1 was downregulated and F-box protein At2g02240 was  
49 upregulated under virus infection. The relatively low number of DEGs identified in Albert compared with  
50 Namikonga (25), which was attributed to a generally weaker transcriptional response in Albert genotype, may  
51 also have reflected in the reduced number of DEGs of VPg-interacting proteins detected in this study. Whereas in  
52 the Namikonga variety, a total of 13 DEGs at the early stage and 4 DEGs at the late stage were identified (Fig  
53 3b). Most of these DEGs of VPg-interacting proteins in Namikonga were observed during the early stages of  
54 infection (2, 5, and 8 DAG), indicating an early gene response of these proteins to viral infection in this variety.  
55 F-box protein At2g02240, tubulin beta-1 chain, nodal modulator 1 isoform X1, and 60S ribosomal protein L9  
56 were upregulated, while fructose-bisphosphate aldolase (cytoplasmic isozyme 1 isoform X1), probable galactinol-  
57 -sucrose galactosyltransferase 6, polyubiquitins, peptidyl-prolyl cis-trans isomerase FKBP20-1 isoform X1,  
58 ribulose bisphosphate carboxylase small subunit (chloroplastic-like), alpha-L-fucosidase-related protein, glycine  
59 cleavage system H protein 3 (mitochondrial), leucine-rich repeat protein kinase-like protein-related and several  
60 uncharacterized proteins, were downregulated.

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## 1 Discussion

2 Understanding the molecular interactions and pathogenesis of CBSVs is critical for developing improved cassava  
3 varieties to control CBSD (32). Like other viruses, CBSVs exploit host cellular pathways through interactions  
4 with host proteins. Identifying these interacting proteins is essential for elucidating infection mechanisms and  
5 cassava resistance to the disease. VPg was selected as bait due to its central role in viral infection as a hub of plant  
6 virus interactions (33). Prior to this study, only eIF(iso)4E and a novel cap-binding protein (nCBP) were identified  
7 as cassava VPg interactors (34). Genes encoding VPg-interacting proteins represent potential targets for  
8 developing recessive resistance or precision genome editing. To our knowledge, this is the first Y2H screen for  
9 cassava proteins interacting with CBSV VPg.

10 A successful Y2H assay requires a high-quality cDNA library, and the cassava library constructed here met this  
11 standard, with robust clone numbers, transformation efficiency, and cell density comparable to previous studies,  
12 and its efficacy was confirmed using  $\beta$ -tubulin as a control bait (35–39). A total of 35 VPg-interacting cassava  
13 proteins were identified in the Y2H assay and also confirmed by one-to-one hybrid assays. These included  
14 chloroplast proteins, metabolic enzymes, housekeeping proteins, and chaperones (Table SI 7), potentially involved  
15 in CBSV replication, plant defense, or symptom development. The presence of chloroplast proteins aligns with  
16 viral targeting of chloroplasts to manipulate host cells (40,41). Host metabolic enzymes likely provide energy and  
17 other multifunctional roles during infection (42,43). Chaperones and folding enzymes support viral replication  
18 and movement complexes (44), while polyubiquitin and ubiquitin-domain proteins highlight the role of the  
19 Ubiquitin/Proteasome system in CBSV infection, consistent with other viruses (45,46).

20 Gene ontology (GO) analysis of the interactors revealed that the predominant molecular function category was  
21 catalytic activity, followed by binding activity. For biological processes, the main categories were cellular and  
22 metabolic processes, while for cellular components, the majority of proteins were associated with cell parts and  
23 organelles. These classifications indicate that CBSV, like other RNA viruses, predominantly interacts with  
24 metabolic enzymes, housekeeping proteins, and other cellular and organellar proteins (42,47–49). Additionally,  
25 GO classification identified nucleic acid binding as a major protein class, which aligns with the primary functions  
26 of the VPg protein in RNA replication, translation, and movement. Furthermore, the interactions of BAG1, DjA6,  
27 SCL8, and PIRPK with VPg were confirmed *in planta* by BiFC analysis in *N. benthamiana*, supporting the  
28 interactions observed in the Y2H assay.

29 The substantial number of VPg-interacting genes perturbed by CBSV infection suggests their involvement in host-  
30 virus interactions. Consistent with the previous finding (25), the resistant Namikonga variety exhibited a more  
31 robust transcriptional response among the VPg interactors, with 15 genes differentially expressed compared to  
32 only 2 in the susceptible Albert variety, indicating a more dynamic regulatory network in Namikonga. Notably,  
33 several defense- and stress-related genes, such as F-box protein At2g02240, tubulin beta-1 chain, and 60S  
34 ribosomal protein L9, were upregulated in Namikonga, while Albert showed limited changes, including  
35 downregulation of BAG1. The predominance of differential expression of genes of VPg interactors during early  
36 infection stages in Namikonga may reflect a response associated with the resistance phenotype. Interestingly,  
37 many downregulated genes in Namikonga were involved in core metabolic processes and protein turnover,  
38 possibly reflecting a strategic reallocation of resources toward defence. BAG1 interacts with Hsp70 and functions

39 at the intersection of protein folding, stress response, and apoptosis regulation; therefore, the downregulation of  
40 BAG1 observed in the susceptible Albert variety may affect stress signalling and apoptotic responses (50,51).

#### 41 **A predictive role of cassava proteins in CBSV pathogenesis (Fig 4).**

42 **Virus multiplication:** Following entry, CBSV likely uses VPg to co-opt host translation machinery, recruiting  
43 proteins such as the 60S ribosomal protein L9, which has also been implicated in viral translation processes in  
44 other systems (52–54). However, these interactions and reliance on host translational machinery may also lead to  
45 virus vulnerability (55). VPg’s interaction with chaperones like Peptidyl-prolyl cis-trans isomerase FKBP20-1  
46 and FKBP16-3, known for roles in protein folding, suggests viral dependence on host folding machinery (56–59).  
47 Yet, these same chaperones may also participate in immune surveillance pathways or protein quality control  
48 mechanisms that limit viral accumulation (60). Similarly, metabolic enzymes like fructose-bisphosphate aldolase  
49 (61), alpha-glucosidase (62,63), L-arabinokinase, and phosphoglucomutase may support viral replication but  
50 could also be redirected by the host to metabolic states unfavourable to viral propagation.

51 **Virus multiplication in organelles:** Chloroplasts are key sites for plant viral replication, and many chloroplast  
52 proteins interact with virus to facilitate its replication and movement. VPg interacted with several chloroplast  
53 membrane-bound proteins, including photosystem I subunits and APO protein 1, which may be involved in viral  
54 replication complex (VRC) formation. However, these interactions may have dual consequences, as chloroplasts  
55 play a key role in plant immunity, particularly in generating reactive oxygen species (ROS), and disruption of  
56 their function could trigger immune signalling pathways (64). Proteins such as plastid-lipid-associated protein 8  
57 may stabilize VRCs or mitigate chloroplast stress, while the 30S ribosomal protein S10 (65) and transcription  
58 factor Scarecrow-like protein 8 might support viral replication and host gene regulation. Chaperones such as  
59 FKBP16-3 and dnaJ A6, which support protein turnover and facilitate access to the Hsp70 system, are likely  
60 critical for viral replication and transport (66). VPg’s interaction with proteins like pheophytinase may affect  
61 chloroplast structure and function, that may cause leaf symptoms as seen in cassava mosaic disease (67). VPg  
62 also interacted with mitochondrial proteins, including CHCH domain-containing protein 10 (68) and the glycine  
63 cleavage system H protein, which may be involved in processes relevant to viral replication in mitochondria.

#### 64 **Virus cell-to-cell and long-distance movement:**

65 Intracellular and systemic movement of CBSV may involve VPg interactions with cytoskeletal, motor and  
66 trafficking proteins like tubulin beta-1 chain, kinesin like protein KIN-14E, and RAS (RAAt Sarcoma)-related  
67 protein RABF1 (RAs-related in Brain F1: motif). Microtubules (69–71) and kinesins (72) provide structural and  
68 motor support for viral trafficking, while Rab-like GTPases mediate membrane trafficking and may support  
69 endosome-based VRC formation (73). Additionally, VPg interactions with heavy metal-associated isoprenylated  
70 plant protein 7 suggest roles in long-distance movement (74), and associations with membrane proteins such as  
71 receptor-like kinase At3g56050 and nodal modulator 1 may facilitate viral RNA export and intercellular spread.

#### 72 **Virus stabilization and protection:**

73 VPg interacts with proteins of the ubiquitin proteasome machinery, which may be involved in functions such as  
74 modification of viral proteins, suppression of silencing, and regulation of effector triggered immunity (ETI). The  
75 VPg interacts with F-box protein, polyubiquitin and proteins containing ubiquitin domain like BAG protein. VPg  
76 interaction with nuclear proteins such as ATRX homolog involved in chromatin remodelling, gene regulation and

77 regulation of cell cycle fate (75), and protein RRC1, which is involved in mRNA splicing and export/import may  
78 aid in viral replication and persistence.

79 The identified interactions between CBSV VPg and cassava host proteins provide insights that help us better  
80 understand the CBSV infection process and resistance mechanisms. These proteins have the potential to be  
81 developed into molecular markers for CBSV resistance following evaluation in cassava breeding segregating  
82 populations.

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4 maintaining cassava plants and Dr. Simon Richardson for providing access to the confocal microscope used in the  
5 BiFC experiment.

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7 **Data availability statement**

8 The excel file containing DEGs data from the studies of Amuge et al. (2017) and Maruthi et al. (2014) are provided  
9 as supplementary materials.

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Figures

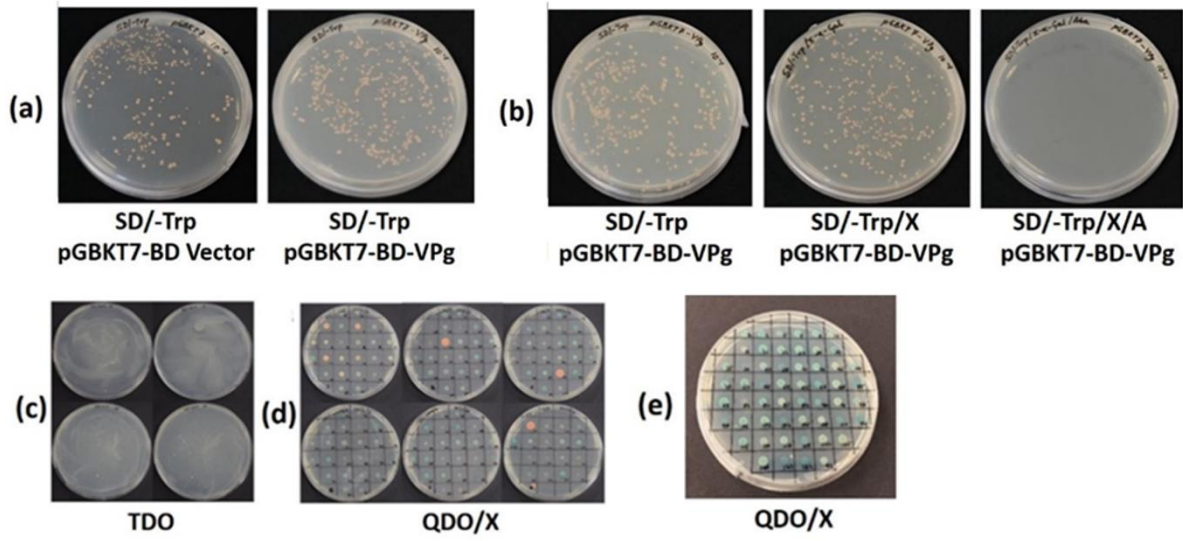


Fig 1.

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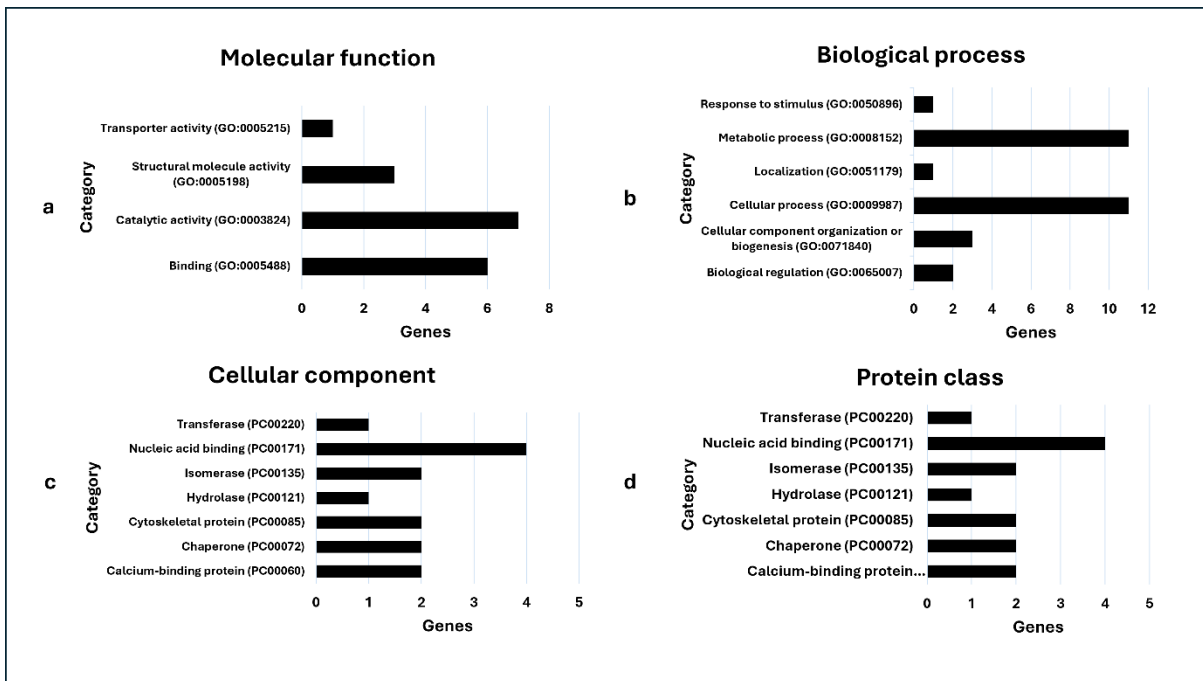
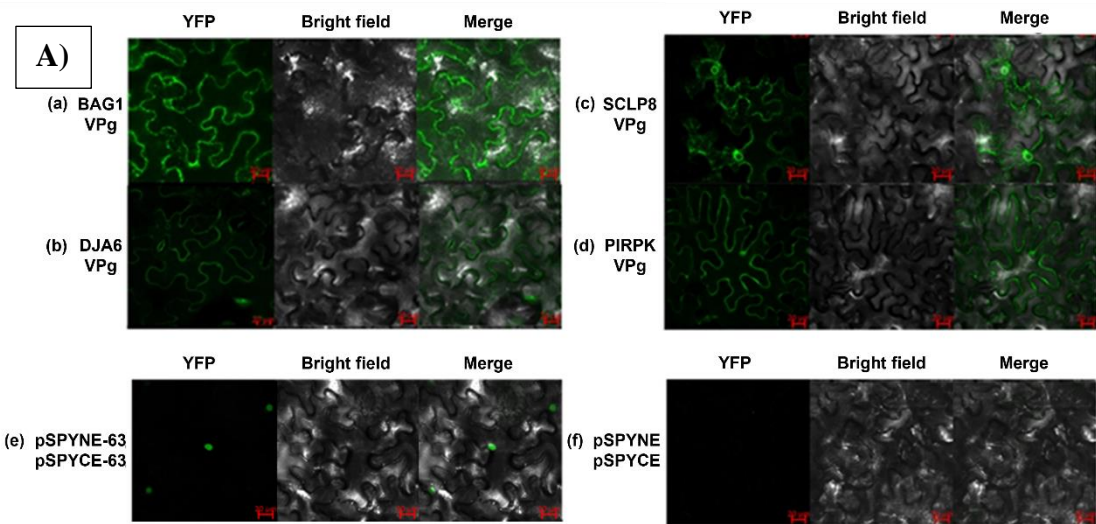
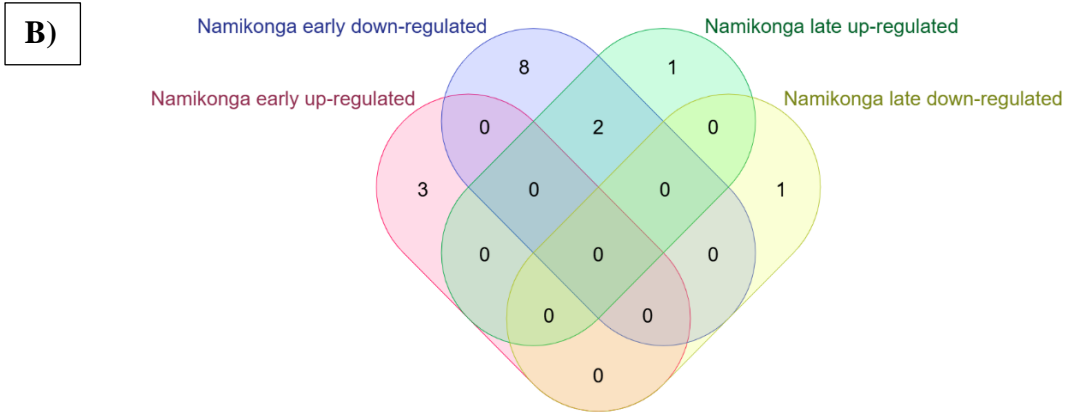


Fig 2.

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**Fig 3.**

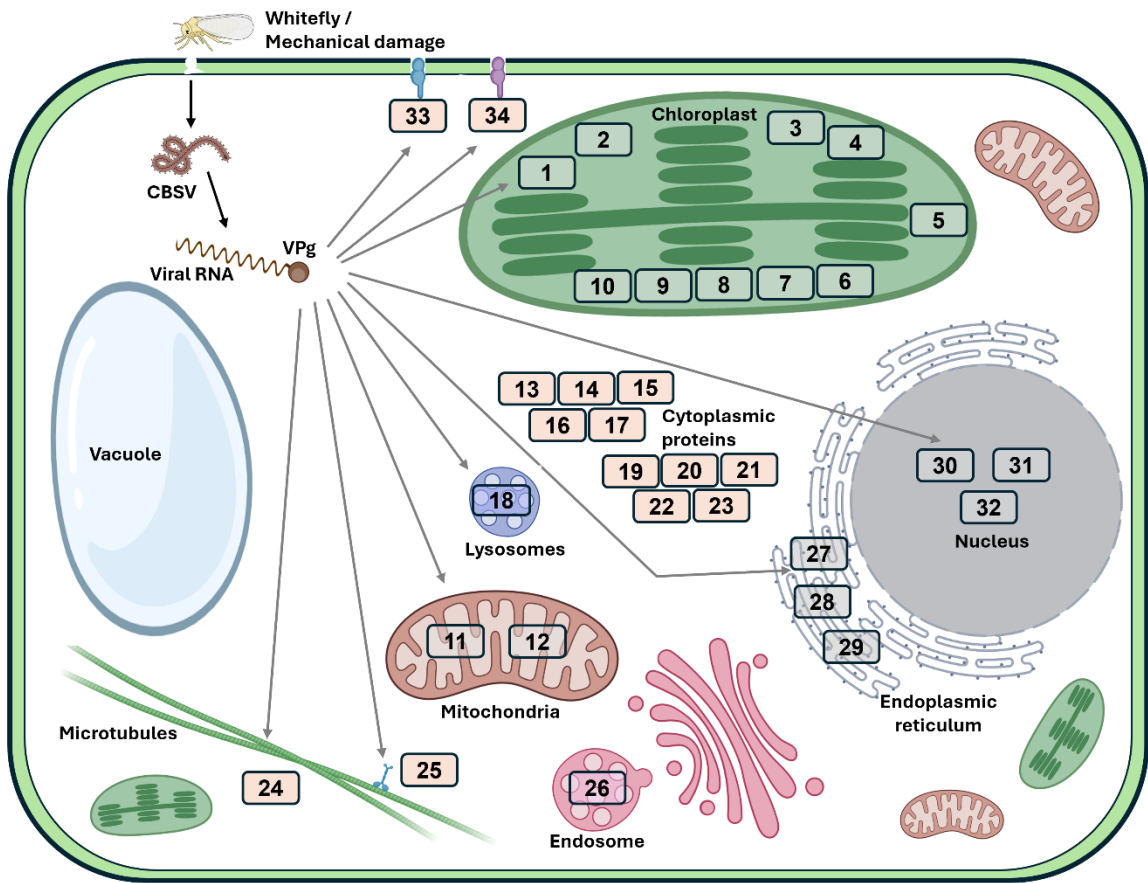


Fig 4.

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### Legends of Figures

38 **Fig 1.** Growth of yeast colonies on different growth medium: (a) Growth of yeast transformed with pGBKT7  
 39 plasmid and pGBKT7-BD-VPg; (b) Growth of yeast colonies transformed with pGBKT7-BD-VPg on SD/-  
 40 Trp, SD/-Trp/X and SD/-Trp/X/A; (c) Growth of colonies on TDO selection media; (d) Growth of colonies  
 41 on QDO/X selection media; (e) Growth of retransformed interacting colonies on QDO/X media.

42 **Fig 2.** Gene ontology based on (a) molecular function; (b) biological process; (c) cellular component; and  
 43 (d) protein class.

44 **Fig 3. (A)** *In-planta* interactions of CBSV VPg protein with cassava proteins analysed by BiFC in leaf  
 45 epidermal cells of *N. benthamiana* plants. Scale bar = 20  $\mu$ m: (a) CBSV VPg with BAG1; (b) CBSV VPg  
 46 with DjA6; (c) CBSV VPg with SCLP8; (d) CBSV VPg with PIRPK; (e) Positive control contains pSPYCE  
 47 and pSPYNE vector containing bZIP63; (f) Negative control contains empty pSPYCE and pSPYNE vector.  
 48 **(B)** Venn diagram showing the overlap of differentially expressed genes (DEGs) in the Namikonga cassava  
 49 variety in response to CBSV infection.

50 **Fig 4.** Schematic depiction of CBSV VPg and cassava protein interacting network in different compartments of  
 51 the cell. The interacting proteins aid in viral replication including transcription, translation, protein turnover and  
 52 movement. The major cassava protein interactions with VPg are as follows. In chloroplast VPg interacts with  
 53 following proteins, 1. Probable plastid-lipid-associated protein 8, 2. Uncharacterised LOC110624715, 3.  
 54 Photosystem I reaction center subunit II, 4. Photosystem I reaction centre subunit V, 5. 30S ribosomal protein  
 55 S10, 6. Scarecrow-like protein 8, 7. APO protein 1, 8. Peptidyl-prolyl cis-trans isomerase FKBP16-3, 9.  
 56 Chaperone protein dnaJ A6, 10. Pheophytinase. The mitochondria associated proteins were 11. Glycine cleavage  
 57 system H protein, mitochondrial-like, 12. Coiled-coil-helix-coiled-coil-helix domain-containing protein 10. The  
 58 cytoplasmic metabolic enzymes and housekeeping proteins were 13. L-arabinokinase, 14. Fructose-bisphosphate  
 59 aldolase, 15. Phosphoglucomutase, 16. Galactinol--sucrose galactosyltransferase 6, 17. Alpha-glucosidase, 18.  
 60 Alpha-L-fucosidase 1, 19. F-box protein At2g02240, 20. BAG 1, 21. Polyubiquitin, 22. Polyubiquitin, 23. Heavy  
 61 metal-associated isoprenylated plant protein 7. The proteins predicted to be associated cytoskeleton and CBSV  
 62 movement were, 24. Tubulin beta-1 chain, 25. Kinesin-like protein KIN-14E, 26. Ras-related protein RABF1.  
 63 The protein associated with CBSV protein synthesis and turnover were, 27. 60S ribosomal protein L9, 28.  
 64 Peptidyl-prolyl cis-trans isomerase FKBP20-1, 29. Uncharacterised LOC110624773. The proteins associated with  
 65 transcription regulation were, 30. Uncharacterised protein LOC110614007, 31. Protein RRC1, 32. Transcriptional

66 regulator ATRX homolog. The plasma membrane associated proteins are 33. Probable inactive receptor-like  
67 protein kinase At3g56050 and 34. Nodal modulator 1 isoform X.

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102 **Table 1.** Cassava proteins that interacted with VPg of CBSV identified by screening cassava cDNA library and  
 103 one-to-one Y2H.

Protein ID	Protein	No of clones
XP_021611143.1	Uncharacterized protein LOC110614007 isoform X1	11
XP_021597072.1	Probable plastid-lipid-associated protein 8, chloroplastic	4
XP_021625673.1	Uncharacterized protein LOC110624715	3
XP_021597173.1	L-arabinokinase isoform X1	3
XP_021619846.1	F-box protein At2g02240	2
XP_021619128.1	<b>BAG family molecular chaperone regulator 1</b>	2
XP_021625915.1	<b>Probable inactive receptor-like protein kinase At3g56050</b>	2
XP_021609720.1	Peptidyl-prolyl cis-trans isomerase FKBP16-3, chloroplastic isoform X1	2
XP_021618306.1	Peptidyl-prolyl cis-trans isomerase FKBP20-1 isoform X1	1
XP_021626007.1	Ras-related protein RABF1 isoform X2	1
XP_021622897.1	Uncharacterized protein LOC110622626 [Manihot esculenta]	1
XP_021615889.1	<b>Chaperone protein dnaJ A6, chloroplastic isoform X1</b>	1
XP_021608524.1	60S ribosomal protein L9	1
XP_021592369.1	<b>Scarecrow-like protein 8</b>	1
XP_021598264.1	Polyubiquitin	1
XP_021626010.1	APO protein 1, chloroplastic isoform X1	1
XP_021607580.1	Kinesin-like protein KIN-14E isoform X2	1
XP_021617386.1	Coiled-coil-helix-coiled-coil-helix domain-containing protein 10, mitochondrial	1
XP_021633848.2	Heavy metal-associated isoprenylated plant protein 7	1
XP_021618731.1	Polyubiquitin	1
XP_021606347.1	Protein RRC1	1
XP_021612733.1	Fructose-bisphosphate aldolase, cytoplasmic isozyme 1 isoform X1	1
XP_021594447.1	Phosphoglucomutase, cytoplasmic	1
XP_021628576.1	Probable galactinol--sucrose galactosyltransferase 6	1
XP_021612506.1	Nodal modulator 1 isoform X1	1
XP_021604665.1	Transcriptional regulator ATRX homolog	1
XP_021595227.1	Photosystem I reaction center subunit II, chloroplastic	1
XP_021597659.1	Uncharacterized protein LOC110603959 isoform X2	1
XP_021593999.1	Glycine cleavage system H protein, mitochondrial	1
XP_021597199.1	Alpha-glucosidase	1
XP_021615435.1	Tubulin beta-1 chain	1
XP_021608810.1	30S ribosomal protein S10, chloroplastic	1
XP_021633973.1	Alpha-L-fucosidase 1	1
XP_021625779.1	Uncharacterized protein LOC110624773	1
XP_021596583.1	Pheophytinase, chloroplastic	1
XP_021614407.1	Photosystem I reaction center subunit V,	1

104 \*Proteins (BAG1, PIRPK, DjA6, & SCL8) analysed by the BIFC assays.

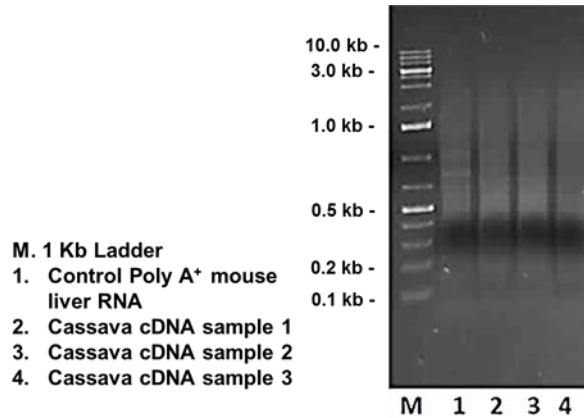
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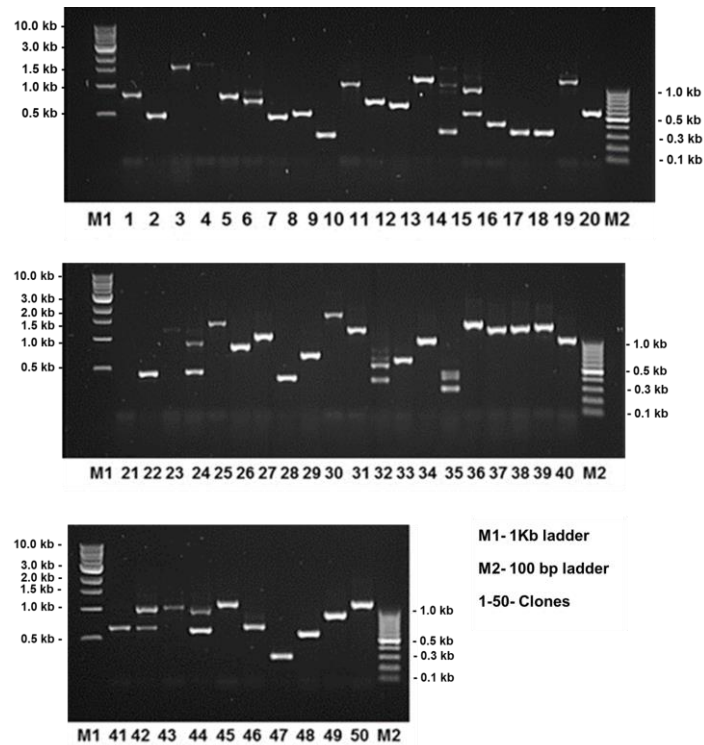
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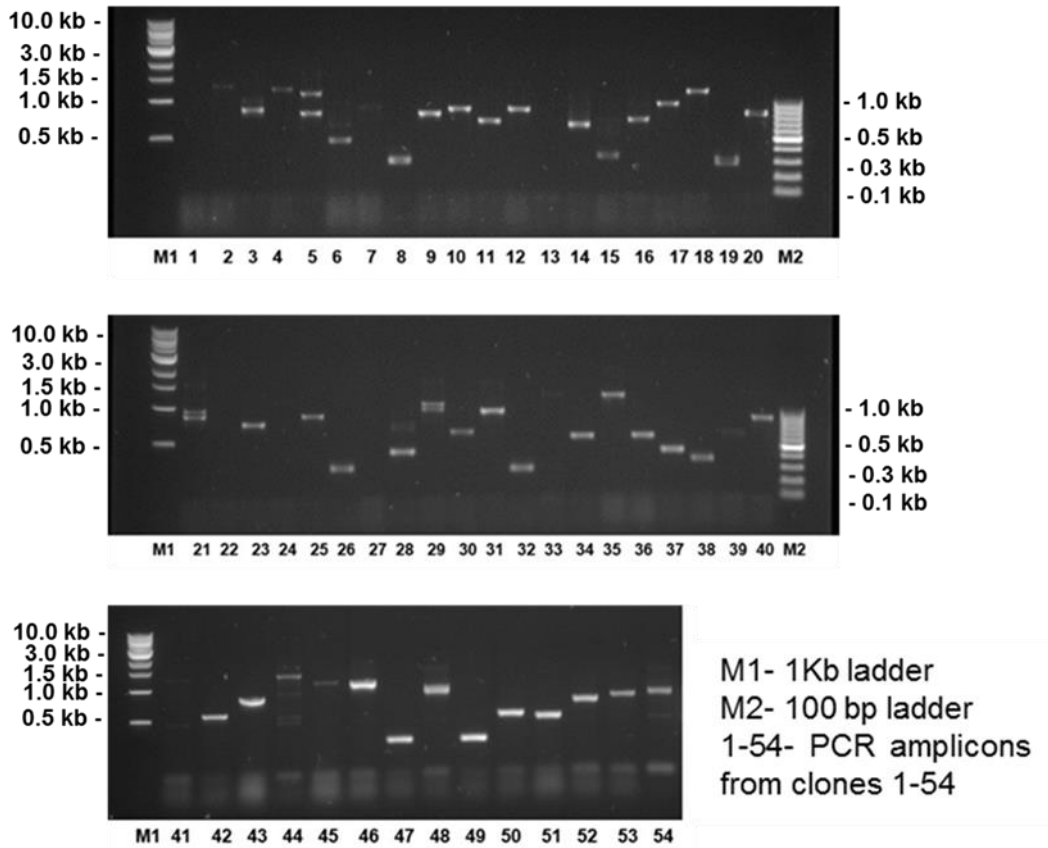
Supplementary Materials:



**Fig SI 1: Electrophoresis of cDNA generated through LD-PCR amplification of mRNA from cassava.** Double stranded cDNA of cassava amplified by LD-PCR were visualised on 1% agarose gel and had a size range between 0.1-3 Kb. The sample bands were clear and bright, the 'smear' was uniform indicating overall, good uniform amplification in LD-PCR and high-quality cDNA generated.



**Fig SI 2: Colony PCR amplification of 50 randomly selected clones from cassava cDNA library.** Colony PCR of 50 random clones revealed the sizes of inserted cDNA in the cassava cDNA library varied from 300 bp to 1800 bp with an average insert size of 765 bp.

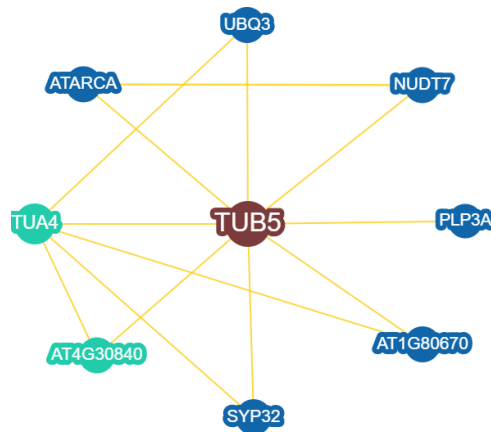


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17 **Fig SI 3: Colony PCR of some randomly selected  $\beta$ -tubulin interacting clones.**

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21 **Fig SI 4: *Arabidopsis* TUB5 protein interactions obtained from the BioGrids database.**

22 TUA4 and AT430840 were identified as interactors (green) of cassava  $\beta$ -tubulin through the Y2H screening.

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**Table SI 1: Primers used to amplify CBSV VPg. Restriction sites are underlined.**

Primer	Sequence	Primer Description	Gene amplified	Amplicon size	T <sub>m</sub> °C
VPg-seq-F	GAGAARGTGATGART GTGAAG	Flanking forward primer to amplify VPg segment	6K2-VPg-Nla-Pro segment	1108 bp	54.3
VPg-seq-R	CCATCATRYTGATAW GTCCA	Flanking reverse primer to amplify VPg segment	6K2-VPg-Nla-Pro segment	1108 bp	54.9
VPg-EcoR1-AS-F	ACG <u>GAA TTC</u> GCA AAG CAC AAG TAT AAT CGG	Specific primer to amplify VPg	VPg	558 bp + 9bp	60.8
VPg-BamH1-AS-R	GAC <u>GGA TCC</u> TTG CTT TTC GAC ACT CTG AT	Specific primer to amplify VPg	VPg	558 bp + 9bp	61.7

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**Table SI 2: Details of primers used in BiFC.**

Primer Name	Sequence	Length (bp)	GC %	T <sub>m</sub> (°C)	Gene specific T <sub>m</sub> (°C)
VPg pSPYNE <i>Bam</i> HI F	CGCCACTAGTGGATCCGCAAAGCACAAATATA ATCGGGAC	40	50	70.6	60.9
VPg pSPYNE <i>Kpn</i> I R	TCCATCCCGGGAGCGGTACCTTGCATCTCAACT GTTTGATCATC	44	52	72.4	60.0
BAG1 pSPYCE <i>Bam</i> HI F	CGCCACTAGTGGATCCATGAAGATGGAGTCAA TGAAGTCA	40	48	69.3	59.9
BAG1 pSPYCE <i>Kpn</i> I R	TACATCCCGGGAGCGGTACCTTAATCAAAAGT TTCCAGTTTGTT	45	44	69.9	58.2
DjA6 pSPYCE <i>Bam</i> HI F	CGCCACTAGTGGATCCATGGCCACTATACCTT GCGG	36	58	75.8	62.2
DjA6 pSPYCE <i>Kpn</i> I R	TACATCCCGGGAGCGGTACCTCATCTTCGACT GCTCACTG	40	57	77.4	59.6
SLP8 pSPYCE <i>Bam</i> HI F	CGCCACTAGTGGATCCATGGCATATGGGTTCTT CGG	36	56	74.6	60.0
SLP8 pSPYCE <i>Kpn</i> I R	TACATCCCGGGAGCGGTACCTTAACGCCAAGC AGATGC	38	58	77.6	58.6
PIRPK pSPYCE <i>Bam</i> HI F	CGCCACTAGTGGATCCATGGACAAGAGAAACT GGAAATTCA	41	46	68.8	60.8
PIRPK pSPYCE <i>Kpn</i> I R	TACATCCCGGGAGCGGTACCTTAGCTAGCATC TGGTGACA	40	55	76.9	58.3

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**Table SI 3: Clone combinations used to investigate cassava and VPg protein-protein interactions.**

Sl. No.	Protein-protein interactions studied	Clones in agroinfiltration mixture
1.	VPg & BAG1	pSPYCE- BAG1 & pSPYNE-VPg
2.	VPg & <i>DjA6</i>	pSPYCE- DjA6 & pSPYNE-VPg
3.	VPg & SCL 8	pSPYCE- SCL8 & pSPYNE-VPg
4.	VPg & PIRPK	pSPYCE- PIRPK & pSPYNE-VPg
5.	Positive control	pSPYCE-63 & pSPYNE-63
6.	Negative control	pSPYCE & pSPYNE

**Table SI 4: Fifty randomly selected cassava proteins that interacted with  $\beta$ -tubulin bait protein in the Y2H assay.**

Clone	Phytozome blast hit	Description
1	Manes.01G059600.1	Aquaporin PIP1-4-related
2	Manes.07G136300.1	Ataxin-2 C-terminal region (PAM2)
3	Manes.07G026200.1	Ras-related protein Rab-1A (RAB1A)
4	Manes.12G067900.1	Glucose-1-phosphate adenylyltransferase small subunit, chloroplastic
5	Manes.18G062200.1	Serine/threonine-protein kinase PEPKR2
6	Manes.05G003500.1	Calvin cycle protein CP12-1, chloroplastic-related
7	Manes.07G056600.1	Tubulin alpha (TUBA)
8	Manes.01G011500.1	Ribulose biphosphate carboxylase small chain 1A, chloroplastic-related
9	Manes.12G097200.1	Photosystem II oxygen-evolving enhancer protein 1 (psbO)
10	Manes.12G097200.1	Photosystem II oxygen-evolving enhancer protein 1 (psbO)
11	Manes.05G191300.1	Plastid transcriptionally active7
12	Manes.16G099000.1	60S Ribosomal protein L18A // subfamily not named
13	Manes.12G047600.1	Wound-inducible basic protein family (Wound_ind)
14	Manes.02G133200.1	Metallothionein (Metallothio_2)
15	Manes.02G111400.1	UPF0548 protein
16	Manes.12G024100.1	Glycine transaminase / Glycine aminotransferase
17	Manes.05G099400.1	Cyclophilin-like peptidyl-prolyl cis-trans isomerase family protein
18	Manes.01G181700.1	Serine--glyoxylate transaminase / SGAT
19	Manes.03G137500.1	Serine/threonine-protein kinase WNK11-related
20	Manes.07G107600.1	Ankyrin repeat-containing protein
21	Manes.03G155600.1	Genomic DNA, chromosome 3, BAC clone:F14O13
22	Manes.03G048700.1	UDP-sulfoquinovose synthase / Sulfite:UDP-glucose sulfotransferase
23	Manes.15G169800.1	Glycine cleavage system H protein 3, mitochondrial
24	Manes.18G003900.1	High mobility group B protein 2-related
25	Manes.12G006200.1	Uncharacterized protein family UPF0016 (UPF0016) // PPR repeat (PPR) // PPR repeat family (PPR_2)
26	Manes.11G072400.1	Glyceraldehyde-3-phosphate dehydrogenase (NADP(+)) (phosphorylating) / Triosephosphate dehydrogenase (NADP+)
27	Manes.05G117400.1	Protein DR1 homolog
28	Manes.04G052000.1	Ethylene insensitive 3-like 1 protein-related
29	Manes.01G011500.1	Ribulose biphosphate carboxylase small chain 1a, chloroplastic-related
30	Manes.03G033900.1	WD40 repeat family // subfamily not named
32	Manes.10G029000.1	Actin-related protein 5 (ACTR5, ARP5, INO80M)
33	Manes.13G040500.1	Large subunit ribosomal protein L23e (RP-L23e, RPL23)
34	Manes.17G066700.1	Light-harvesting complex II chlorophyll a/b binding protein 1 (LHCB1)
35	Manes.09G154900.1	Small subunit ribosomal protein S3Ae (RP-S3Ae, RPS3A)
36	Manes.17G066800.1	Light-harvesting complex II chlorophyll a/b binding protein 1 (LHCB1)
37	Manes.14G056600.1	Methionine sulfoxide reductase // subfamily not named
38	Manes.01G011500.1	Ribulose biphosphate carboxylase small chain 1A, chloroplastic-related
39	Manes.03G042300.1	Chlorophyll A/B binding protein // subfamily not named LinksB M
40	Manes.09G065000.1	E3 ubiquitin-protein ligase RFWD3 [EC:6.3.2.19] (RFWD3)
41	Manes.07G002700.1	Large subunit ribosomal protein L6e (RP-L6e, RPL6)
42	Manes.16G073000.1	Profilin (PFN)
43	Manes.07G143600.1	-
44	Manes.13G132100.1	-
45	Manes.15G086700.1	-
46		Chromosome02:15032710..15033167 (+ strand)
47		Chromosome18:9125869..9126004 (- strand)
48	No hit	

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96**Table SI 5: List of proteins interacted with Arabidopsis TUB5 based on BioGrids database.**

<b>Interactor</b>	<b>Experimental Evidence</b>
AT4G30840 - Transducin/WD40 domain-containing protein	Affinity Capture-MS
TUA4 - Tubulin alpha-4 chain	Co-purification
AT1G80670 - Rae1-like protein	Affinity Capture-MS
UBQ3 - Polyubiquitin 3	Affinity Capture-MS
NUDT7 - Nudix hydrolase 7	Reconstituted Complex
ATARCA-Guanine nucleotide-binding protein subunit beta-like protein A	Protein-Fragment
	Complementation Assay
SYP32 - Syntaxin-32	Affinity Capture-MS
PLP3A - Phosducin-like protein 3-like protein	Y2H

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**Table SI 6: Log<sub>2</sub> fold-change values of genes under control and CBSV-infected conditions.**

<b>Late response-Albert</b>						
<b>NCBI Protein ID</b>	<b>NCBI Name</b>	<b>Phytozome V6.1</b>	<b>Phytozome V4.1</b>	<b>45 DAG-Nam</b>	<b>54 DAG-Nam</b>	<b>1 YAG-Kal</b>
XP_021619128.1	BAG family molecular chaperone regulator 1	Manes.01G066900	cassava4.1_013107m.g	-2.00		
XP_021619846.1	F-box protein At2g02240	Manes.08G105600	cassava4.1_012511m.g			4.92
<b>Early response-Namikonga/Kalawi</b>						
<b>NCBI Protein ID</b>	<b>NCBI Name</b>	<b>Phytozome V6.1</b>	<b>Phytozome V4.1</b>	<b>2 DAG-Nam</b>	<b>5 DAG-Nam</b>	<b>8 DAG-Nam</b>
XP_021612733.1	Fructose-bisphosphate aldolase, cytoplasmic isozyme 1 isoform X1	Manes.05G202700	cassava4.1_010561m.g		-3.54	-1.93
XP_021628576.1	Probable galactinol--sucrose galactosyltransferase 6	Manes.01G175500	cassava4.1_002003m.g	-2.78		
XP_021618731.1	Polyubiquitin	Manes.07G019300	cassava4.1_009650m.g		-1.95	
XP_021618306.1	Peptidyl-prolyl cis-trans isomerase FKBP20-1 isoform X1	Manes.10G039600	cassava4.1_016955m.g		-1.41	
XP_043809748.1	Ribulose bisphosphate carboxylase small subunit, chloroplastic-like	Manes.01G011500	cassava4.1_017170m.g			-1.41
XP_021625915.1	Probable inactive receptor-like protein kinase At3g56050	Manes.10G009600	cassava4.1_003119m.g	-1.01	-1.32	
XP_021597659.1	Uncharacterized protein LOC110603959 isoform X2	Manes.16G126300	cassava4.1_013834m.g		-1.26	
XP_021622897.1	Uncharacterized protein LOC110622626 [Manihot esculenta]	Manes.09G133000	cassava4.1_031293m.g		-1.22	
XP_021633973.1	Alpha-L-fucosidase 1	Manes.14G031700	cassava4.1_006636m.g			-1.15
XP_021593999.1	Glycine cleavage system H protein, mitochondrial	Manes.15G169800	cassava4.1_017913m.g		-1.14	
XP_021608524.1	60S ribosomal protein L9	Manes.11G113000	cassava4.1_016807m.g		1.00	
XP_021612506.1	Nodal modulator 1 isoform X1	Manes.05G014200	cassava4.1_000458m.g		1.06	
XP_021615435.1	Tubulin beta-1 chain	Manes.06G147900	cassava4.1_007632m.g	1.35	1.38	
<b>Late response-Namikonga/Kalawi</b>						
<b>NCBI Protein ID</b>	<b>NCBI Name</b>	<b>Phytozome V6.1</b>	<b>Phytozome V4.1</b>	<b>45 DAG-Nam</b>	<b>54 DAG-Nam</b>	<b>1 YAG-Kal</b>
XP_021598264.1	Polyubiquitin	Manes.17G035300	cassava4.1_012545m.g			-1.42
XP_021612733.1	Fructose-bisphosphate aldolase, cytoplasmic isozyme 1 isoform X1	Manes.05G202700	cassava4.1_010561m.g			1.15

XP_021619846.1	F-box protein At2g02240	Manes.08G105600	cassava4.1_012511m.g		3.07	
XP_021628576.1	Probable galactinol--sucrose galactosyltransferase 6	Manes.01G175500	cassava4.1_002003m.g		3.07	

**Table SI 7: VPg interacting important cassava proteins, their location function and probable role in CBSV infection.**

Sl. No	Protein	Location	Function	Known viral associated functions	Reference	Probable role in CBSD
1.	The 30S ribosomal protein S10, chloroplastic-like	Chloroplast	Protein synthesis	<ul style="list-style-type: none"> <li>• Translational need of viruses</li> </ul>	Lohmus, Varjosalo and Makinen, 2015, Li, 2019	Viral protein synthesis or/and impair host translation in chloroplast
2.	Peptidyl-prolyl cis-trans isomerase FKBP16-3, chloroplastic	Chloroplast	Protein turnover, catalyses cis/trans isomerization leading to folding of protein into active confirmation.	<ul style="list-style-type: none"> <li>• PPIases identified as a potential interactor of P3N-PIPO protein of <i>Soybean mosaic virus</i>.</li> <li>• Many viruses use PPIases of the host for their own protein modification.</li> </ul>	Song, et al., 2016, Kromina, et al., 2008	Chaperone activity of the viral protein produced in chloroplast
3.	Molecular chaperone DnaJ (DjA6)	Chloroplast	Co-chaperon of Hsp70.	<ul style="list-style-type: none"> <li>• Host DnaJ-Like proteins act as important susceptibility factors and are required for PVY infection in tobacco plants.</li> </ul>	Hofius, et al., 2007	Utilised to access the various Hsp70 associated function required for viral replication, infection, and transport.
4.	Plastid-lipid-associated protein 8	Chloroplast - thylakoid membranes and plastoglobules	Accumulate under various abiotic and biotic stress and has a protective role.		Leitner-Dagan et al., 2006, Singh and McNellis, 2011	Required to relieve the virus induced stress in chloroplast and/or maintenance of VRCs.
5.	Photosystem I reaction center subunit V, chloroplastic and Photosystem I reaction center subunit II chloroplastic like	Chloroplast	Photosynthesis.	<ul style="list-style-type: none"> <li>• Chloroplast-localized photosystem I PSI-K protein have an antiviral role as their silencing enhances virus accumulation.</li> </ul>	Jimenez et al., 2006	Change in availability these proteins may be affecting the photosynthesis in chloroplast thus causing chlorosis
6.	APO protein 1, chloroplastic	Chloroplast	Involved in assembly of light harvesting complexes.		Amann et al., 2004	Change in availability these proteins may be affecting the photosynthesis in chloroplast thus causing chlorosis.

7.	Pheophytinase	Chloroplast	Chlorophyll breakdown is observed during leaf senescence.	<ul style="list-style-type: none"> <li>• The chlorophyll degradation by enzymes like pheophytinase was implicated to be reason for mosaic symptoms development in cassava mosaic disease.</li> </ul>	Liu et al., 2014	Chlorotic symptoms development.
8	Fructose-bisphosphate aldolase	Cytoplasm	Enzyme involved in glycolysis, part of carbohydrate metabolism	<ul style="list-style-type: none"> <li>• Similar metabolic enzyme glyceraldehyde 3-phosphate dehydrogenase is required for regulation of +/- RNA synthesis in the VRC.</li> </ul>	Wang and Ahlquist, 2008	Functions inside VRC.
9	L-arabinokinase	Cytoplasm	Nucleotide sugars metabolism leading to synthesis of polysaccharides.			Functions inside VRC.
10	Phosphoglucomutase	Cytoplasm	Starch synthesis.	<ul style="list-style-type: none"> <li>• A defect in carbohydrate metabolism due to defective phosphoglucomutase ameliorates symptom.</li> </ul>	Handford and Carr, 2007	Altered starch accumulation would lead to disease symptom in CBSV infected plants.
11	Probable galactinol-sucrose galactosyltransferase 6	Cytoplasm	Enzyme involved in synthesis of raffinose and myo-inositol.	<ul style="list-style-type: none"> <li>• Raffinose is involved in biotic stress signalling and its accumulation is found to induce defence gene expression.</li> <li>• Raffinose are also involved in protection of plant cell from oxidative damage by scavenging hydroxyl radicals.</li> </ul>	Kim et al., 2008 Nishizawa et al., 2008 Sengupta et al., 2015 Chaouch and Noctor, 2010	Raffinose for membrane stabilisation or myo-inositol for abolishing the salicylic acid-dependent cell death and pathogen defence responses.
12	Alpha-glucosidase	Cytoplasm	Starch breakdown, cellulose biosynthesis, and morphogenesis.	<ul style="list-style-type: none"> <li>• <math>\alpha</math>-Glucosidase inhibitors have been used as antiviral agents.</li> </ul>	Burn et al., 2002, Gillmor et al., 2002 Mehta et al., 1998	Quality control of the proteins in ER.
13	Alpha-L-fucosidase	Cytoplasm	Degradation of glycoproteins and hemicellulose.		Voiniciuc et al., 2018, Takahisa and Rumi, 2011, Augur et al., 2002	Loosening of the cell wall required for its growth and restructuring of the membrane replication complex.

14	BAG proteins	Cytoplasm	Co-chaperon of Hsp70, apoptosis, cell signalling, and transcriptional regulation and deciding the fate of proteins.	<ul style="list-style-type: none"> <li>• BAG1 based antiapoptotic activity induced by <i>Herpes simplex virus</i>.</li> </ul>	Kabbage and Dickman, 2008; Alberti, Essar and Hohfeld, 2003	To associate with the chaperone and utilise ubiquitin proteasome system.
15	Heavy metal-associated isoprenylated plant protein 7-like	Cytoplasm	Metallochaperones, proteins involved in transport of metallic ions inside the cell.	<ul style="list-style-type: none"> <li>• HIPPS involved in heavy metal homeostasis and detoxification mechanisms like cadmium. Cadmium is known to block virus invasion in plants.</li> <li>• Transcriptional response to various biotic and abiotic stresses.</li> </ul>	Carr and Murphy, 2002; Ghosroy et al., 2002	Overcome the cadmium mediated viral resistance and also to overcome the general biotic stress associated with viral infection.
16	Beta-tubulin	Cytoplasm	Part of the microtubule	<ul style="list-style-type: none"> <li>• Involved in transport of plant viral RNA, virus particles.</li> <li>• Regulate many processes including viral replication complex synthesis.</li> </ul>	Boyko et al., 2000 Naghavi and Walsh, 2017	Transport of viral RNA or virus.
17	Kinesin-like protein KIN-14E	Cytoplasm	Microtubule-based motor proteins that are involved in transportation of various cargoes, including protein complexes, mRNAs, and membranous organelles.		Li, Xu and Chong, 2012 Peña and Heinlein, 2012, Niehl et al., 2013	Motor proteins to transport RNA, protein, and other cargo to VRC and other locations.
18	Ras-related protein RABF1 (ARA6)	Peripheral membrane of the endosome.	Rab-like GTP-ase involved in membrane trafficking, salt and osmotic stress tolerance, flowering	<ul style="list-style-type: none"> <li>• The endocytic pathway for viral intracellular movement as seen in <i>Potato mop-top virus</i>.</li> <li>• They are involved in intracellular membrane trafficking and are critical host</li> </ul>	Tsutsui, Nakano and Ueda, 2015 Haupt et al., 2005 Xu and Nagy, 2016	Intracellular membrane trafficking and VRC assembly and formation.

			and starch and sugar homeostasis.	factors that assist in VRC assembly and formation for <i>Tomato bushy stunt virus</i> (TBSV).		
19	Transcriptional regulator ATRX ( $\alpha$ -thalassemia X-linked intellectual disability)	Nucleus	Chromatin remodelling proteins, histone deposition at telomeres, induce transcriptional gene silencing or regulate the cell cycle fate.	<ul style="list-style-type: none"> <li>Restrict gene expression from DNA viruses and induce latency but their role in RNA virus remains obscure.</li> </ul>	Duc et al., 2017 Cabral, Oh and Knipe, 2018	Not known.
20	Protein RRC1-like (Reduced red-light responses in cry1cry2 background 1),	Nucleus	Orthologue of the putative human splicing factor SR140, involved in splicing of pre-mRNA and post splicing activities like mRNA decay, export/import, or translation.		Shikata, et al., 2012, Long and Caceres, 2009	Splicing and/or membrane trafficking activities.
21	Polyubiquitin and proteins containing ubiquitin domain	Cytoplasm		<ul style="list-style-type: none"> <li>Ubiquitin modification of cellular and viral proteins together with actions of the 26S proteasome are indispensable for the regulation of viral infection.</li> </ul>	Alcaide-Loridan and Jupin 2012	UPS machinery to cause infection
22	F-box protein	Cytoplasm	Components of the SCF (SKp1, Cullin, F-box protein) ubiquitin-ligase complexes, which mediates polyubiquitination.	<ul style="list-style-type: none"> <li>The viruses are known to use the host UPS for promoting infection.</li> </ul>	Correa et al., 2013	F-box protein to induce polyubiquitination of target proteins to access the UPS machinery in host cells.
23	Probable inactive receptor-like protein kinase	Transmembrane proteins		<ul style="list-style-type: none"> <li>Leucine-rich repeat receptor-like protein was found to assist <i>Bamboo mosaic virus</i> cell-to-cell movement.</li> </ul>	Chen et al., 2017	RNA transport or defence regulation.

(Leucine-rich repeat  
receptor-like  
protein kinase)

24	Nodal modulator 1	Plasma membrane	Antagonistic effect on nodal signalling in humans.	Perea et al., 2017	RNA movement.
25	The Coiled-coil- helix-coiled-coil- helix domain- containing protein 10, mitochondrial- like	Mitochondria	Implicated in mitochondrial cristae stability and oxidative phosphorylation in humans.	Genin et al., 2016, Bannwarth et al., 2014, Martherus et al., 2010	Replication in mitochondrial VRCs.
26	Glycine cleavage system H protein, mitochondrial-like	Mitochondria	Glycine catabolism.		Replication in mitochondrial VRCs.

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