



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

Sumesh M. Kakkunnath¹ · Sophie Bouvaine¹ · Siji P. Kavi¹ · M. N. Maruthi¹

Received: 15 October 2025 / Accepted: 19 January 2026 / Published online: 19 March 2026
© The Author(s) 2026

Abstract

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

Keywords Cassava brown steak virus · VPg · Yeast two hybrid · Protein-protein interaction · cDNA library · chloroplast

Introduction

Cassava (*Manihot esculenta* Crantz) grown mainly for its starchy tuberous roots is consumed by approximately 800 million people worldwide [1, 2]. It is a food security crop in many countries of Sub-Saharan Africa (SSA) and is gaining importance as an industrial crop especially for its use in the production of starch. However, the emergence of two viral diseases, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) has severely affected cassava production and food security in SSA [3]. With the recent spread of CMD to East Asia and the expected spread of CBSD to West Africa, they have been considered the greatest threat to cassava production globally [4, 5]. More than half of the cassava plants grown in Africa are affected by

CMD and CBSD that results in annual production losses of more than US\$ 3 billion [6, 7]. CBSD can reduce production up to 70% of the total root yield in susceptible varieties [8]. Analysis of whole genomes of several viruses isolated from CBSD-infected plants revealed the presence of two distinct virus species: Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV) (genus *Ipomovirus*, family *Potyviridae*) [9–11], which are collectively called cassava brown streak viruses (CBSVs). CBSV is transmitted by the insect vector, whitefly, *Bemisia tabaci* [12], although field transmission is predominantly by infected stem cuttings.

Plant host factors play a crucial role in the infection process of CBSV, as is the case with many plant viruses. Successful infection depends on complex interactions between viral and host factors that facilitate viral genome replication, as well as cell-to-cell and long-distance movement. Viruses reshape the intracellular environment by interacting with pro-viral host factors, co-opting essential cellular processes, and interfering with host antiviral defences. On the contrary, interactions with antiviral host factors determine the effectiveness of plant defence mechanisms, such

✉ M. N. Maruthi
m.n.maruthi@gre.ac.uk

¹ Present address: Natural Resources Institute (NRI),
University of Greenwich, Chatham Maritime, Kent, United
Kingdom

as the hypersensitive response and post-transcriptional gene silencing, and influence the virus's ability to overcome these defences [13, 14]. Some of these interactions may also contribute to disease symptom development, either directly or through collateral effects unrelated to virus replication [15]. Due to the constraints of the viral genome size, and associated limited coding capacity, the viral proteins are multifunctional and have evolved to target various host factors [16]. The viral protein VPg, is essential for infectivity. It binds to many plant proteins as it is a hub controlling processes involved in the multiplication and spread of viruses [17, 18].

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

In this study, we used the CBSV VPg protein as bait to identify interacting host proteins from a newly synthesized cassava cDNA library. We further validated the *in-planta* interactions of four selected candidates in *Nicotiana benthamiana* using a bimolecular fluorescence complementation (BiFC) assay for validating the identified interactors. To assess the relevance of these interactions during infection, we examined the expression patterns of VPg-interacting genes among the DEGs identified in previous studies of resistant (Namikonga) and susceptible (Albert) cassava varieties following viral infection [25, 27]. The VPg-interacting host proteins identified in this work provide new insights into the CBSV infection process and represent potential targets for enhancing cassava resistance to the virus.

Materials and methods

Plant materials and virus source

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

RNA isolation and Y2H cDNA library construction

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

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

Y2H assays

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

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

Screening for VPg-interacting proteins

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

For Y2H screening, Y2HGold cells expressing the VPg bait were mated with the cassava cDNA library in strain Y187. Serial dilutions of the mated culture were plated on SD/–Trp, SD/–Leu, and SD/–Leu/–Trp (DDO) media to estimate the number of clones screened. Positive interactions were selected on QDO/X (SD/–Ade/–His/–Leu/–Trp supplemented with X- α -Gal). Colonies growing on QDO/X plates were analyzed by colony PCR, and inserts were sequenced. Clones yielding multiple amplicons were re-streaked on QDO/X plates until single inserts were obtained. Sequence data were used to identify corresponding genes and proteins using Phytozome and NCBI databases. Redundant clones were removed, and one representative clone per gene was selected for further analysis. Subcellular localization was predicted using MultiLoc2 [30], and gene ontology analysis was performed using the PANTHER database.

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

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

Bimolecular fluorescence complementation (BiFC)

To confirm the *in-planta* interaction between cassava genes and the VPg protein, a BiFC analysis was performed in *Nicotiana benthamiana* using a subset of four genes selected based on their presumed importance in CBSV

infection process. The four cassava genes were: Bcl-2 (B-cell lymphoma 2)-associated athano gene 1 (BAG 1, XP_021619128.1), chaperone protein dnaJ A6 (DjA6, XP_021615889.1), Probable inactive receptor-like protein kinase At3g56050 (PIRPK, XP_021625915.1), and Scarecrow-like protein 8 (SCL8, XP_021592369.1). The BiFC vectors pSPYNE and pSPYCE (N- and C-terminal YFP fragments) were obtained from Prof. Dr. Jörg Kudla (University of Münster, Germany). BiFC constructs were generated by cloning cassava genes into pSPYCE and VPg into pSPYNE using the In-Fusion HD cloning kit (Clontech, USA). Genes were PCR-amplified with primers (Table SI 2), purified, and inserted into BamHI-linearized pSPYNE or KpnI-linearized pSPYCE vectors. Agrobacterium clones were prepared, and working suspensions of paired constructs (Table SI 3) were used for agroinfiltration into *Nicotiana benthamiana* leaves following [31]. Leaf segments (5 × 5 mm) were collected three days post-infiltration and imaged for fluorescence using a Zeiss LSM 880 confocal microscope at 20× magnification. YFP reconstitution was monitored using an argon ion laser at 488 nm, with excitation at 514 nm and emission at 520–540 nm.

Expression analysis of VPg-interacting cassava genes

To explore the relationship between VPg-interacting cassava proteins and CBSVs resistance, gene expression profiles were analysed in previously published RNA-seq datasets. Differential gene expression data listed in the studies (Amuge et al., 2017; Maruthi et al., 2014) which analysed CBSV responses in two cassava genotypes with contrasting resistance phenotypes: Albert (CBSV-susceptible) and Namikonga (CBSV-resistant, also known as Kaleso), were retrieved (Supplementary materials excel file 1 & 2) and analysed. Log₂ fold-change (log₂FC) values comparing CBSV-infected and mock-inoculated plants were extracted for all genes encoding VPg-interacting proteins identified in this study. In these studies, expression data encompassed multiple infection stages, which was further grouped here as early (2, 5, and 8 days after grafting [DAG]) and late (45 DAG and 1 year after grafting [YAG]) time points. DEGs were selected based on a |log₂FC| > 1 threshold. The number and direction of DEGs were determined separately for each genotype and time point, and results were summarized to compare transcriptional responses between resistant and susceptible varieties.

Results

Cassava cDNA library preparation and VPg bait quality assessment

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

Y2H screening of CBSV VPg interactors

The mated culture of the bait strain (pGBKT7-BD-VPg) with the cassava cDNA library yielded 136 colonies on 49

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

Gene ontology of VPg-interacting cassava proteins

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

In-planta interactions of cassava proteins through BiFC

The *in-planta* interactions of four cassava proteins; BAG1, DjA6, SCL8, and PIRPK, selected based on their presumed

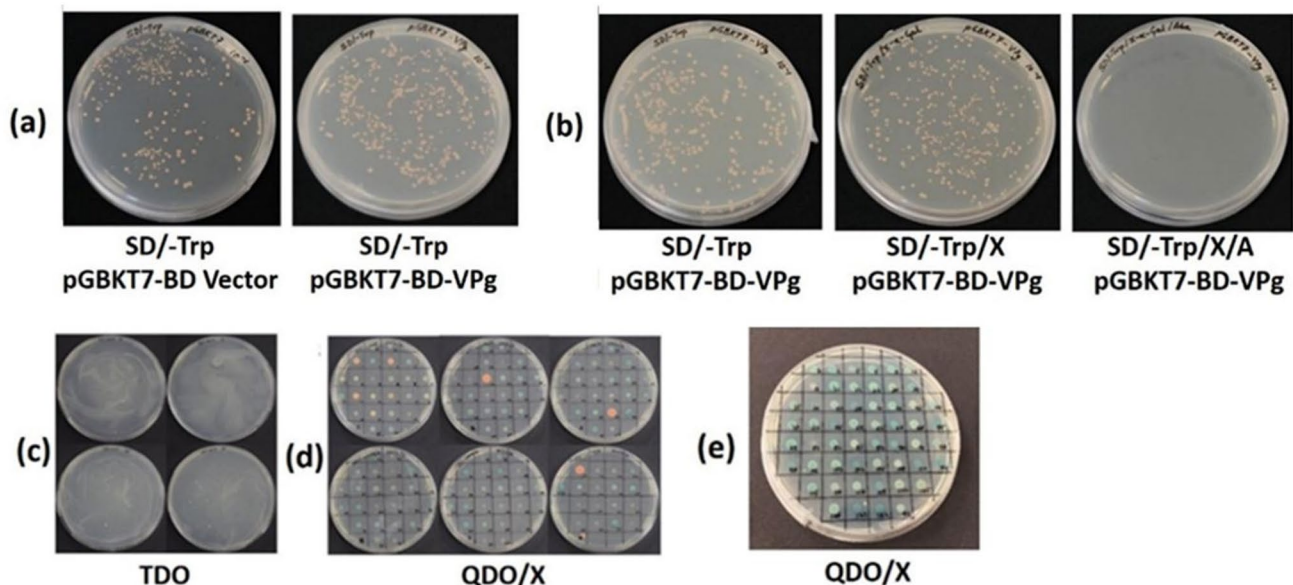


Fig. 1 Growth of yeast colonies on different growth medium: (a) Growth of yeast transformed with pGBKT7 plasmid and pGBKT7-BD-VPg; (b) Growth of yeast colonies transformed with pGBKT7-BD-VPg on SD/-Trp, SD/-Trp/X and SD/-Trp/X/A; (c) Growth of

colonies on TDO selection media; (d) Growth of colonies on QDO/X selection media; (e) Growth of retransformed interacting colonies on QDO/X media.

Table 1 Cassava proteins that interacted with VPg of CBSV identified by screening cassava cDNA library and 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	BAG family molecular chaperone regulator 1	2
XP_021625915.1	Probable inactive receptor-like protein kinase At3g56050	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	Chaperone protein dnaJ A6, chloroplastic isoform X1	1
XP_021608524.1	60 S ribosomal protein L9	1
XP_021592369.1	Scarecrow-like protein 8	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	30 S 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

*Proteins (BAG1, PIRPK, DjA6, & SCL8) analysed by the BiFC assays

roles in the CBSV infection process, were validated by BiFC analysis in *Nicotiana benthamiana*. Fluorescence signals were detected for all four cassava proteins (Fig. 3A), confirming their *in-planta* interactions with VPg in *N. benthamiana*. The fluorescence signals were observed at both the cell periphery, cytoplasm and in the nucleus. Strong fluorescence signals indicating the BAG1–VPg interaction were predominantly observed in the cytoplasm, although the speckled pattern may be indicating fluorescence originating from plastids. Weak fluorescence signals corresponding to the DjA6–VPg interaction were detected in the cytoplasm. Fluorescence signals for the SCL8–VPg interaction were observed in both the cytoplasm and the nucleus, with nuclear signals suggesting localization at the nuclear membrane. Fluorescence signals for the PIRPK–VPg interaction

were also observed in the periphery which might indicate cytoplasm or membrane-based interaction.

Expression analysis of the identified VPg-interacting cassava proteins

To investigate the relationship between the VPg-interacting cassava proteins and the observed differential resistance to CBSVs, we examined their gene expression profiles in resistant Namikonga and susceptible Albert cassava variety. Log₂ fold-change values under both control and CBSVs-infected conditions were extracted from two previously conducted studies [25, 27]. Across both studies, 22 of the 36 VPg-interacting genes identified were found to be differentially expressed between virus infected and uninfected

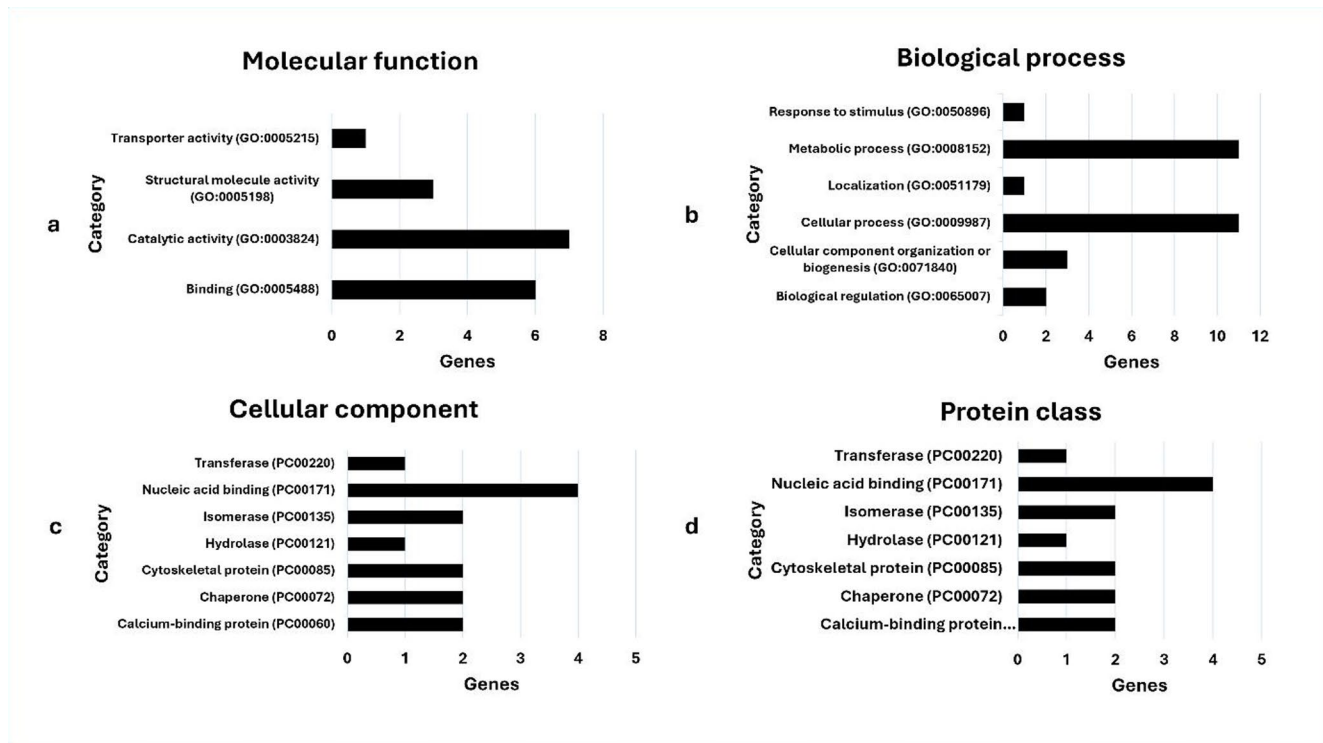


Fig. 2 Gene ontology based on (a) molecular function; (b) biological process; (c) cellular component; and (d) protein class

plants when considering both the Albert and Namikonga varieties combined. After applying a $|\log_2 \text{fold change}| > 1$ threshold, this number was reduced to 16 genes (Table SI 6); of these, 2 DEGs were identified in Albert and 15 in Namikonga. We further classified these DEGs by infection stage into early time points (2, 5, and 8 DAG) and late time points (45 and 54 DAG, and 1 YAG). In the Albert variety, no early stage DEGs were found but at the late stage (45 DAG and 1 YAG, respectively) BAG family molecular chaperone regulator 1 was downregulated and F-box protein At2g02240 was upregulated under virus infection. The relatively low number of DEGs identified in Albert compared with Namikonga [25], which was attributed to a generally weaker transcriptional response in Albert genotype, may also have reflected in the reduced number of DEGs of VPg-interacting proteins detected in this study. Whereas in the Namikonga variety, a total of 13 DEGs at the early stage and 4 DEGs at the late stage were identified (Fig. 3B). Most of these DEGs of VPg-interacting proteins in Namikonga were observed during the early stages of infection (2, 5, and 8 DAG), indicating an early gene response of these proteins to viral infection in this variety. F-box protein At2g02240, tubulin beta-1 chain, nodal modulator 1 isoform X1, and 60 S ribosomal protein L9 were upregulated, while fructose-bisphosphate aldolase (cytoplasmic isozyme 1 isoform X1), probable galactinol–sucrose galactosyltransferase 6, polyubiquitins, peptidyl-prolyl cis-trans isomerase FKBP20-1

isoform X1, ribulose bisphosphate carboxylase small subunit (chloroplastic-like), alpha-L-fucosidase-related protein, glycine cleavage system H protein 3 (mitochondrial), leucine-rich repeat protein kinase-like protein-related and several uncharacterized proteins, were downregulated.

Discussion

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

A successful Y2H assay requires a high-quality cDNA library, and the cassava library constructed here met this standard, with robust clone numbers, transformation

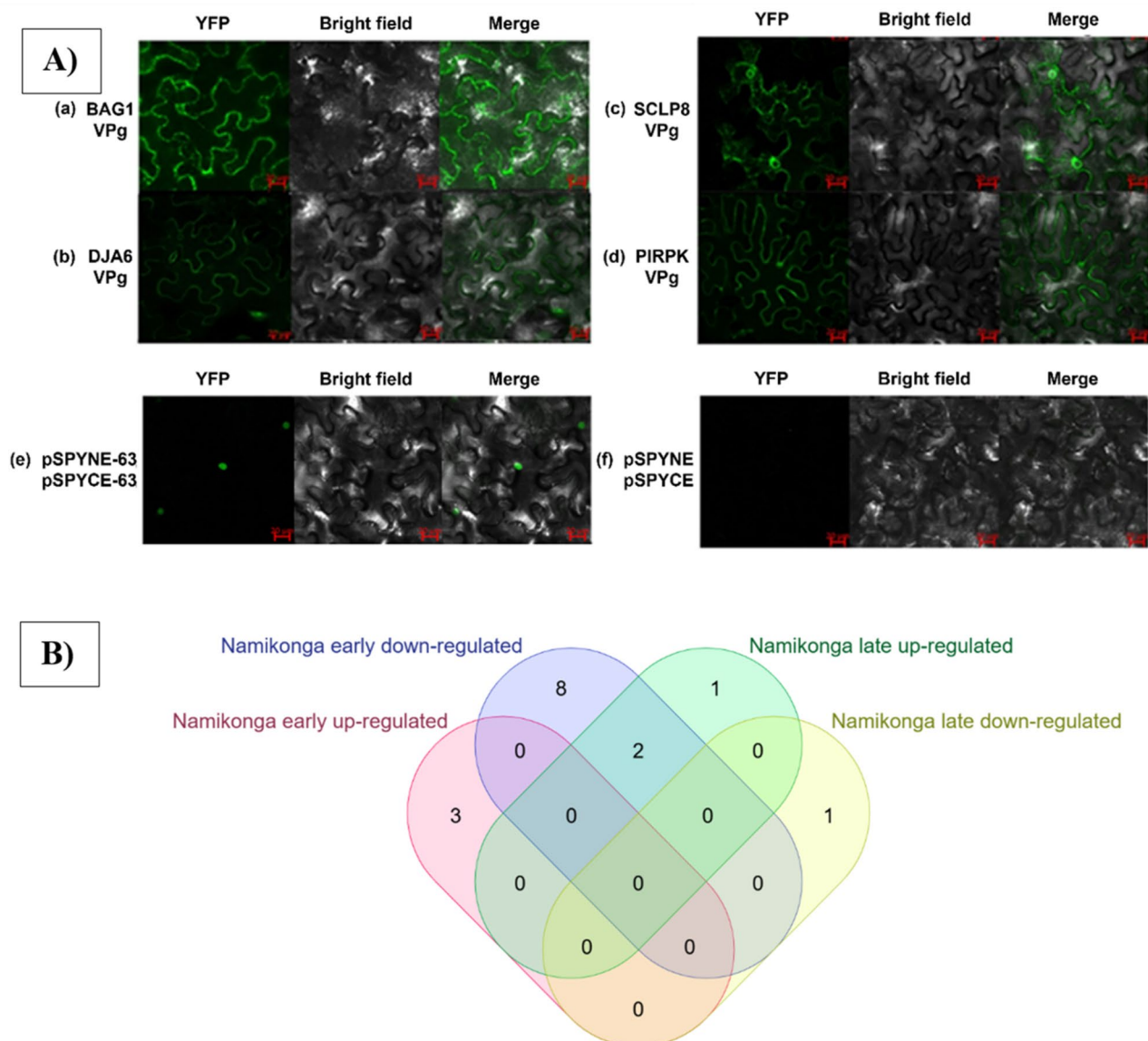


Fig. 3 (A) *In-planta* interactions of CBSV VPg protein with cassava proteins analysed by BiFC in leaf epidermal cells of *N. benthamiana* plants. Scale bar = 20 μ m: (a) CBSV VPg with BAG1; (b) CBSV VPg with DJA6; (c) CBSV VPg with SCLP8; (d) CBSV VPg with PIRPK; (e) Positive control contains pSPYCE and pSPYNE vector

containing bZIP63; f) Negative control contains empty pSPYCE and pSPYNE vector. (B) Venn diagram showing the overlap of differentially expressed genes (DEGs) in the Namikonga cassava variety in response to CBSV infection

efficiency, and cell density comparable to previous studies, and its efficacy was confirmed using β -tubulin as a control bait [35–39]. A total of 35 VPg-interacting cassava proteins were identified in the Y2H assay and also confirmed by one-to-one hybrid assays. These included chloroplast proteins, metabolic enzymes, housekeeping proteins, and chaperones (Table SI 7), potentially involved in CBSV replication, plant defense, or symptom development. The presence of chloroplast proteins aligns with viral targeting of chloroplasts to manipulate host cells [40, 41]. Host metabolic enzymes likely provide energy and other multifunctional roles during infection [42, 43]. Chaperones and folding enzymes support

viral replication and movement complexes [44], while polyubiquitin and ubiquitin-domain proteins highlight the role of the Ubiquitin/Proteasome system in CBSV infection, consistent with other viruses [45, 46].

Gene ontology (GO) analysis of the interactors revealed that the predominant molecular function category was catalytic activity, followed by binding activity. For biological processes, the main categories were cellular and metabolic processes, while for cellular components, the majority of proteins were associated with cell parts and organelles. These classifications indicate that CBSV, like other RNA viruses, predominantly interacts with metabolic enzymes,

housekeeping proteins, and other cellular and organellar proteins [42, 47–49]. Additionally, GO classification identified nucleic acid binding as a major protein class, which aligns with the primary functions of the VPg protein in RNA replication, translation, and movement. Furthermore, the interactions of BAG1, DjA6, SCL8, and PIRPK with VPg were confirmed *in planta* by BiFC analysis in *N. benthamiana*, supporting the interactions observed in the Y2H assay.

The substantial number of VPg-interacting genes perturbed by CBSV infection suggests their involvement in host-virus interactions. Consistent with the previous finding [25], the resistant Namikonga variety exhibited a more robust transcriptional response among the VPg interactors, with 15 genes differentially expressed compared to only 2 in the susceptible Albert variety, indicating a more dynamic regulatory network in Namikonga. Notably, several defense- and stress-related genes, such as F-box protein At2g02240, tubulin beta-1 chain, and 60 S ribosomal protein L9, were upregulated in Namikonga, while Albert showed limited changes, including downregulation of BAG1. The predominance of differential expression of genes of VPg interactors during early infection stages in Namikonga may reflect a response associated with the resistance phenotype. Interestingly, many downregulated genes in Namikonga were involved in core metabolic processes and protein turnover, possibly reflecting a strategic reallocation of resources toward defence. BAG1 interacts with Hsp70 and functions at the intersection of protein folding, stress response, and apoptosis regulation; therefore, the downregulation of BAG1 observed in the susceptible Albert variety may affect stress signalling and apoptotic responses [50, 51].

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

Virus multiplication Following entry, CBSV likely uses VPg to co-opt host translation machinery, recruiting proteins such as the 60 S ribosomal protein L9, which has also been implicated in viral translation processes in other systems [52–54]. However, these interactions and reliance on host translational machinery may also lead to virus vulnerability [55]. VPg's interaction with chaperones like Peptidyl-prolyl cis-trans isomerase FKBP20-1 and FKBP16-3, known for roles in protein folding, suggests viral dependence on host folding machinery [56–59]. Yet, these same chaperones may also participate in immune surveillance pathways or protein quality control mechanisms that limit viral accumulation [60]. Similarly, metabolic enzymes like fructose-bisphosphate aldolase [61], alpha-glucosidase [62, 63], L-arabinokinase, and phosphoglucumutase may support

viral replication but could also be redirected by the host to metabolic states unfavourable to viral propagation (Fig. 4)

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

Virus cell-to-cell and long-distance movement

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

Virus stabilization and protection

VPg interacts with proteins of the ubiquitin proteasome machinery, which may be involved in functions such as modification of viral proteins, suppression of silencing, and regulation of effector triggered immunity (ETI). The VPg

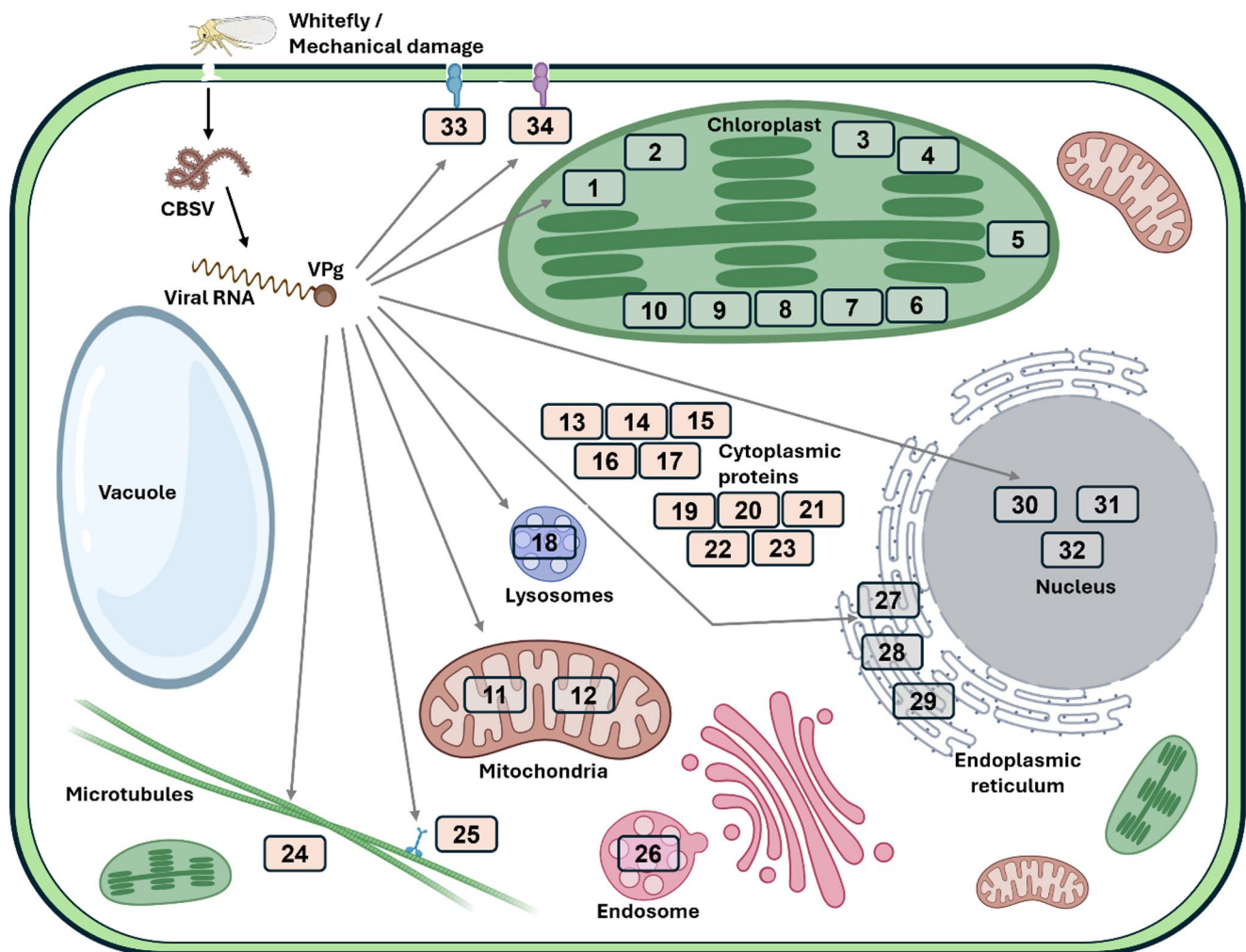


Fig. 4 Schematic depiction of CBSV VPg and cassava protein interacting network in different compartments of the cell. The interacting proteins aid in viral replication including transcription, translation, protein turnover and movement. The major cassava protein interactions with VPg are as follows. In chloroplast VPg interacts with following proteins, 1. Probable plastid-lipid-associated protein 8, 2. Uncharacterised LOC110624715, 3. Photosystem I reaction center subunit II, 4. Photosystem I reaction centre subunit V, 5. 30S ribosomal protein S10, 6. Scarecrow-like protein 8, 7. APO protein 1, 8. Peptidyl-prolyl cis-trans isomerase FKBP16-3, 9. Chaperone protein dnaJ A6, 10. Pheophytinase. The mitochondria associated proteins were 11. Glycine cleavage system H protein, mitochondrial-like, 12. Coiled-coil-helix-coiled-coil-helix domain-containing protein 10. The cytoplasmic metabolic enzymes and housekeeping proteins were 13. L-arabinokinase, 14. Fructose-bisphosphate aldolase, 15. Phosphoglu-

comutase, 16. Galactinol--sucrose galactosyltransferase 6, 17. Alpha-glucosidase, 18. Alpha-L-fucosidase 1, 19. F-box protein At2g02240, 20. BAG 1, 21. Polyubiquitin, 22. Polyubiquitin, 23. Heavy metal-associated isoprenylated plant protein 7. The proteins predicted to be associated cytoskeleton and CBSV movement were, 24. Tubulin beta-1 chain, 25. Kinesin-like protein KIN-14E, 26. Ras-related protein RABF1. The protein associated with CBSV protein synthesis and turnover were, 27. 60S ribosomal protein L9, 28. Peptidyl-prolyl cis-trans isomerase FKBP20-1, 29. Uncharacterised LOC110624773. The proteins associated with transcription regulation were, 30. Uncharacterised protein LOC110614007, 31. Protein RRC1, 32. Transcriptional regulator ATRX homolog. The plasma membrane associated proteins are 33. Probable inactive receptor-like protein kinase At3g56050 and 34. Nodal modulator 1 isoform X

interacts with F-box protein, polyubiquitin and proteins containing ubiquitin domain like BAG protein. VPg interaction with nuclear proteins such as ATRX homolog involved in chromatin remodelling, gene regulation and regulation of cell cycle fate [75], and protein RRC1, which is involved in mRNA splicing and export/import may aid in viral replication and persistence.

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

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1007/s00705-0>

26-06571-y.

Acknowledgements This work was carried out as part of the Projects AURG/2/141 and AURG II-1-060-2016 funded to the Natural Resources Institute by the African Union Commission (AUC). We thank Natalie Morley for assistance in maintaining cassava plants and Dr. Simon Richardson for providing access to the confocal microscope used in the BiFC experiment.

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

Declarations

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Hood ASC, Shackelford GE, Christie AP, Usieta HO, Martin PA, Sutherland WJ (2023) A systematic map of cassava farming practices and their agricultural and environmental impacts using new ontologies: Agri-ontologies 1.0. *Ecol Solut Evid* 4(2):e12249
- Liu J, Zheng Q, Ma Q, Gadidasu KK, Zhang P (2011) Cassava Genetic Transformation and its Application in Breeding^F. *J Integr Plant Biol* 53(7):552–569
- Rey C, Vanderschuren H (2017) Cassava Mosaic and Brown Streak Diseases: Current Perspectives and Beyond. *Annu Rev Virol* 4(1):429–452
- Legg J, Somado EA, Barker I, Beach L, Ceballos H, Cuellar W et al (2014) A global alliance declaring war on cassava viruses in Africa. *Food Secur* 6(2):231–248
- Robson F, Hird DL, Boa E (2024) Cassava brown streak: A deadly virus on the move. *Plant Pathol* 73(2):221–241
- Patil BL, Legg JP, Kanju E, Fauquet CM (2015) Cassava brown streak disease: a threat to food security in Africa. *J Gen Virol* 96(5):956–968
- Roderick J, Hillocks RJH, Midatharahally N, Maruthi MNM (2015) Post-harvest impact of cassava brown streak disease in four countries in eastern Africa. *Food Chain* 5(1):116–122
- Hillocks RJ, Raya MD, Mtunda K, Kiozia H (2001) Effects of Brown Streak Virus Disease on Yield and Quality of Cassava in Tanzania. *J Phytopathol* 149(7–8):389–394
- Inoue-Nagata AK, Jordan R, Kreuze J, Li F, López-Moya JJ, Mäkinen K et al (2022) ICTV virus taxonomy profile: potyviridae 2022: This article is part of the ICTV virus taxonomy profiles collection. *J Gen Virol* 103(5) [Internet]. [cited 2025 May 26]. Available from: <https://www.microbiologyresearch.org/content/journal/jgv/10.1099/jgv.0.001738>
- Mbanzibwa DR, Tian YP, Tugume AK, Mukasa SB, Tairo F, Kyamanywa S et al (2009) Genetically distinct strains of Cassava brown streak virus in the Lake Victoria basin and the Indian Ocean coastal area of East Africa. *Arch Virol* 154(2):353–359
- Winter S, Koerbler M, Stein B, Pietruszka A, Paape M, Butgereitt A (2010) Analysis of cassava brown streak viruses reveals the presence of distinct virus species causing cassava brown streak disease in East Africa. *J Gen Virol* 91(5):1365–1372
- Maruthi MN, Hillocks RJ, Mtunda K, Raya MD, Muhanna M, Kiozia H et al (2005) Transmission of *Cassava brown streak virus* by *Bemisia tabaci* (Gennadius). *J Phytopathol* 153(5):307–312
- Ershova NM, Kamarova KA, Sheshukova EV, Komarova TV (2025) Cellular Partners of Tobamoviral Movement Proteins. *Int J Mol Sci* 26(1):400
- Garcia-Ruiz H (2019) Host factors against plant viruses. *Mol Plant Pathol* 20(11):1588–1601
- Whitham SA, Wang Y (2004) Roles for host factors in plant viral pathogenicity. *Curr Opin Plant Biol* 7(4):365–371
- Ahlquist P, Noueiry AO, Lee WM, Kushner DB, Dye BT (2003) Host Factors in Positive-Strand RNA Virus Genome Replication. *J Virol* 77(15):8181–8186
- Dunoyer P, Thomas C, Harrison S, Revers F, Maule A (2004) A Cysteine-Rich Plant Protein Potentiates *Potyvirus* Movement through an Interaction with the Virus Genome-Linked Protein VPg. *J Virol* 78(5):2301–2309
- Rajamäki ML, Valkonen JPT (2002) Viral Genome-Linked Protein (VPg) Controls Accumulation and Phloem-Loading of a Potyvirus in Inoculated Potato Leaves. *Mol Plant-Microbe Interactions* 15(2):138–149
- Cuadrado AF, Van Damme D (2024) Unlocking protein–protein interactions in plants: a comprehensive review of established and emerging techniques. Janda M (ed). *J Exp Bot* 75(17):5220–36
- MacFarlane SA, Uhrig JF (2008) Yeast two-hybrid assay to identify host–virus interactions. In: Foster GD, Johansen IE, Hong Y, Nagy PD (ed). *Plant virology protocols* [Internet]. Walker JM (ed). *Methods Mol Biol* 451: 649–72. Totowa, NJ: Humana Press; [cited 2025 May 26]. Available from: http://link.springer.com/10.1007/978-1-59745-102-4_44
- Xu K, Nagy D (2010) Dissecting Virus-Plant Interactions Through Proteomics Approaches. *Curr Proteom* 7(4):316–327
- Matioli CC, Melotto M (2018) A Comprehensive Arabidopsis Yeast Two-Hybrid Library for Protein-Protein Interaction Studies: A Resource to the Plant Research Community. *Mol Plant-Microbe Interactions* 31(9):899–902
- Liu Y, Liu WW, Li L, Francis F, Wang XF (2023) Transcriptome analysis reveals different response of resistant and susceptible rice varieties to rice stripe virus infection. *J Integr Agric* 22(6):1750–1762
- Zanardo LG, De Souza GB, Alves MS (2019) Transcriptomics of plant–virus interactions: a review. *Theor Exp Plant Physiol* 31(1):103–125
- Amuge T, Berger DK, Katari MS, Myburg AA, Goldman SL, Ferguson ME (2017) A time series transcriptome analysis of cassava (*Manihot esculenta* Crantz) varieties challenged with Ugandan cassava brown streak virus. *Sci Rep* 7(1):9747
- Anjanappa RB, Mehta D, Okoniewski MJ, Szabelska-Beręsewicz A, Gruijsse W, Vanderschuren H (2018) Molecular insights into *Cassava brown streak virus* susceptibility and resistance by profiling of the early host response. *Mol Plant Pathol* 19(2):476–489
- Maruthi MN, Bouvaine S, Tufan HA, Mohammed IU, Hillocks RJ (2014) Transcriptional response of virus-infected cassava and identification of putative sources of resistance for cassava brown streak disease. Fang DD (ed). *PLoS ONE* 9(5):e96642
- Mohammed IU, Abarshi MM, Muli B, Hillocks RJ, Maruthi MN (2012) The Symptom and Genetic Diversity of Cassava Brown

- Streak Viruses Infecting Cassava in East Africa. *Adv Virol* 2012:1–10
29. Otti G, Bouvaine S, Kimata B, Mkamillo G, Kumar PL, Tomlins K et al (2016) High-throughput multiplex real-time PCR assay for the simultaneous quantification of DNA and RNA viruses infecting cassava plants. *J Appl Microbiol* 120(5):1346–1356
 30. Blum T, Briesemeister S, Kohlbacher O (2009) MultiLoc2: integrating phylogeny and Gene Ontology terms improves subcellular protein localization prediction. *BMC Bioinformatics* 10(1):274
 31. Waadt R, Schlücking K, Schroeder JI, Kudla J (2014) Protein fragment bimolecular fluorescence complementation analyses for the in vivo study of protein-protein interactions and cellular protein complex localizations. In: Sanchez-Serrano JJ, Salinas J (eds). *Methods Mol Biol* 1062:629–58. *Arabidopsis Protocols* [Internet]. Totowa, NJ: Humana Press; [cited 2025 May 26]. Available from: https://link.springer.com/10.1007/978-1-62703-580-4_33
 32. Mero HR, Lyantagaye SL, Bongcam-Rudloff E (2021) Why has permanent control of cassava brown streak disease in Sub-Saharan Africa remained a dream since the 1930s? *Infect Genet Evol* 94:105001
 33. Jiang J, Laliberté JF (2011) The genome-linked protein VPg of plant viruses—a protein with many partners. *Curr Opin Virol* 1(5):347–354
 34. Gomez MA, Lin ZD, Moll T, Chauhan RD, Hayden L, Renninger K et al (2019) Simultaneous CRISPR/Cas9-mediated editing of cassava *eIF 4E* isoforms *nCBP -1* and *nCBP -2* reduces cassava brown streak disease symptom severity and incidence. *Plant Biotechnol J* 17(2):421–434
 35. Cao S, Yan L (2013) Construction of a high-quality yeast two-hybrid (Y2H) library and its application in identification of interacting proteins with key vernalization regulator TaVRN-A1 in wheat. *BMC Res Notes* 6(1):81
 36. Dharmaraj K, Cui W, Rikkerink EHA, Templeton MD (2019) Construction of a kiwifruit yeast two-hybrid cDNA library to identify host targets of the *Pseudomonas syringae* pv. *actinidiae* effector AvrPto5. *BMC Res Notes* 12(1):63
 37. Gao X, Jia R, Wang M, Zhu D, Chen S, Lin M et al (2014) Construction and identification of a cDNA library for use in the yeast two-hybrid system from duck embryonic fibroblast cells post-infected with duck enteritis virus. *Mol Biol Rep* 41(1):467–475
 38. Mahajan S, Sharma GK, Matura R, Subramaniam S, Mohapatra JK, Pattnaik B (2015) Construction and characterization of yeast two-hybrid cDNA library derived from LFBK cell line. *Biologicals* 43(3):202–208
 39. Xu Y, Zhou J, Liu Q, Li K, Zhou Y (2020) Construction and characterization of a high-quality cDNA library of *Cymbidium faberi* suitable for yeast one- and two-hybrid assays. *BMC Biotechnol* 20(1):4
 40. Bhattacharyya D, Chakraborty S (2018) Chloroplast: the Trojan horse in plant–virus interaction. *Mol Plant Pathol* 19(2):504–518
 41. Bwalya J, Kim KH (2023) The Crucial Role of Chloroplast-Related Proteins in Viral Genome Replication and Host Defense against Positive-Sense Single-Stranded RNA Viruses. *Plant Pathol J* 39(1):28–38
 42. Huang YW, Hu CC, Lin NS, Hsu YH (2012) Unusual roles of host metabolic enzymes and housekeeping proteins in plant virus replication. *Curr Opin Virol* 2(6):676–682
 43. Nagy PD, Lin W (2020) Taking over Cellular Energy-Metabolism for TBSV Replication: The High ATP Requirement of an RNA Virus within the Viral Replication Organelle. *Viruses* 12(1):56
 44. Verchot J (2012) Cellular chaperones and folding enzymes are vital contributors to membrane bound replication and movement complexes during plant RNA virus infection. *Front Plant Sci* [Internet]. [cited 2025 May 26] Available from: <http://journal.frontiersin.org/article/10.3389/fpls.2012.00275/abstract>
 45. Dubiella U, Serrano I (2021) The Ubiquitin Proteasome System as a Double Agent in Plant-Virus Interactions. *Plants* 10(5):928
 46. Langin G, González-Fuente M, Üstün S (2023) The Plant Ubiquitin-Proteasome System as a Target for Microbial Manipulation. *Annu Rev Phytopathol* 61(1):351–375
 47. Chaturvedi S, Seo JK, Rao ALN (2016) Functionality of host proteins in Cucumber mosaic virus replication: GAPDH is obligatory to promote interaction between replication-associated proteins. *Virology* 494:47–55
 48. Kaido K, Abe A, Mine M, Hyodo K, Taniguchi T, Taniguchi H et al (2014) GAPDH-A recruits a plant virus movement protein to cortical virus replication complexes to facilitate viral cell-to-cell movement. *Nagy PD (ed). PLoS Pathog* 10(11):e1004505
 49. Prasanth KR, Chuang C, Nagy PD (2017) Co-opting ATP-generating glycolytic enzyme PGK1 phosphoglycerate kinase facilitates the assembly of viral replicase complexes. *Dinesh-Kumar SP (ed). PLOS Pathog* 13(10):e1006689
 50. Jiang H, Liu X, Xiao P, Wang Y, Xie Q, Wu X et al (2023) Functional insights of plant bcl-2-associated anahogene (BAG) proteins: Multi-taskers in diverse cellular signal transduction pathways. *Front Plant Sci* 14:1136873
 51. Nawkar GM, Maibam P, Park JH, Woo SG, Kim CY, Lee SY et al (2017) In silico study on Arabidopsis BAG gene expression in response to environmental stresses. *Protoplasma* 254(1):409–421
 52. Beyer AR, Bann DV, Rice B, Pultz IS, Kane M, Goff SP et al (2013) Nucleolar Trafficking of the Mouse Mammary Tumor Virus Gag Protein Induced by Interaction with Ribosomal Protein L9. *J Virol* 87(2):1069–1082
 53. Cerna H, Černý M, Habánová H, Šafářová D, Abushamsiya K, Navrátil M et al (2017) Proteomics offers insight to the mechanism behind *Pisum sativum* L. response to pea seed-borne mosaic virus (PSBMV). *J Proteom* 153:78–88
 54. Löhmus A, Varjosalo M, Mäkinen K (2016) Protein composition of 6K2-induced membrane structures formed during *Potato virus A* infection. *Mol Plant Pathol* 17(6):943–958
 55. Li Y, Dong W, Shi Y, Deng F, Chen X, Wan C et al (2016) Rabies virus phosphoprotein interacts with ribosomal protein L9 and affects rabies virus replication. *Virology* 488:216–224
 56. Kaul A, Stauffer S, Berger C, Pertel T, Schmitt J, Kallis S et al (2009) Essential role of cyclophilin A for hepatitis C virus replication and virus production and possible link to polyprotein cleavage kinetics. *Rice CM (ed). PLoS Pathog* 5(8):e1000546
 57. Nagy PD, Pogany J (2012) The dependence of viral RNA replication on co-opted host factors. *Nat Rev Microbiol* 10(2):137–149
 58. Okamoto T, Nishimura Y, Ichimura T, Suzuki K, Miyamura T, Suzuki T et al (2006) Hepatitis C virus RNA replication is regulated by FKBP8 and Hsp90. *EMBO J* 25(20):5015–5025
 59. Watashi K, Ishii N, Hijikata M, Inoue D, Murata T, Miyanari Y et al (2005) Cyclophilin B is a functional regulator of hepatitis C virus RNA polymerase. *Mol Cell* 19(1):111–122
 60. Chang H, Lee C, Chang C, Jan F (2022) FKBP-type peptidyl-prolyl *cis-trans* isomerase interacts with the movement protein of tomato leaf curl New Delhi virus and impacts viral replication in *Nicotiana benthamiana*. *Mol Plant Pathol* 23(4):561–575
 61. Gao L, Shen WT, Yan P, Tuo DC, Li XY, Zhou P (2012) A set of host proteins interacting with papaya ringspot virus NIa-Pro protein identified in a yeast two-hybrid system. *Acta Virol* 56(1):25–30
 62. Courageot MP, Frenkiel MP, Duarte Dos Santos C, Deubel V, Despres P (2000) α -Glucosidase Inhibitors Reduce Dengue Virus Production by Affecting the Initial Steps of Virion Morphogenesis in the Endoplasmic Reticulum. *J Virol* 74(1):564–572
 63. Qu X, Pan X, Weidner J, Yu W, Alonzi D, Xu X et al (2011) Inhibitors of endoplasmic reticulum α -glucosidases potentially suppress hepatitis C virus virion assembly and release. *Antimicrob Agents Chemother* 55(3):1036–1044

64. Jiménez I, López L, Alamillo JM, Valli A, García JA (2006) Identification of a Plum pox virus CI-interacting protein from chloroplast that has a negative effect in virus infection. *Mol Plant Microbe Interact* 19(3):350–358
65. Wang R, Du Z, Bai Z, Liang Z (2017) The interaction between endogenous 30S ribosomal subunit protein S11 and cucumber mosaic virus LS2b protein affects viral replication, infection and gene silencing suppressor activity. Wong SM (ed). *PLOS ONE* 12(8):e0182459
66. Du Y, Zhao J, Chen T, Liu Q, Zhang H, Wang Y et al (2013) Type I J-domain NbMIP1 proteins are required for both tobacco mosaic virus infection and plant innate immunity. Dinesh-Kumar SP (ed). *PLoS Pathog* 9(10):e1003659
67. Liu J, Yang J, Bi H, Zhang P (2014) Why mosaic? Gene expression profiling of *African cassava mosaic virus* s-infected cassava reveals the effect of chlorophyll degradation on symptom development. *J Integr Plant Biol* 56(2):122–132
68. Genin EC, Plutino M, Bannwarth S, Villa E, Cisneros-Barroso E, Roy M et al (2016) *CHCHD 10* mutations promote loss of mitochondrial cristae junctions with impaired mitochondrial genome maintenance and inhibition of apoptosis. *EMBO Mol Med* 8(1):58–72
69. Boyko V, Hu Q, Seemanpillai M, Ashby J, Heinlein M (2007) Validation of microtubule-associated Tobacco mosaic virus RNA movement and involvement of microtubule-aligned particle trafficking. *Plant J* 51(4):589–603
70. Ashby J, Boutant E, Seemanpillai M, Sambade A, Ritzenthaler C, Heinlein M (2006) Tobacco Mosaic Virus Movement Protein Functions as a Structural Microtubule-Associated Protein. *J Virol* 80(17):8329–8344
71. McLean BG, Zupan J, Zambryski PC (1995) Tobacco mosaic virus movement protein associates with the cytoskeleton in tobacco cells. *Plant Cell* 7(12):2101–2114
72. Jouvenet N, Monaghan P, Way M, Wileman T (2004) Transport of African Swine Fever Virus from Assembly Sites to the Plasma Membrane Is Dependent on Microtubules and Conventional Kinesin. *J Virol* 78(15):7990–8001
73. Xu K, Nagy PD (2016) Enrichment of phosphatidylethanolamine in viral replication compartments via co-opting the endosomal rab5 Small GTPase by a positive-strand RNA virus. *PLoS Biol* 14(10):e2000128
74. Cowan GH, Roberts AG, Jones S, Kumar P, Kalyandurg PB, Gil JF et al (2018) Potato mop-top virus co-opts the stress sensor HIP26 for long-distance movement. *Plant Physiol* 176(3):2052–2070
75. Lukashchuk V, Everett RD (2010) Regulation of ICP0-Null Mutant Herpes Simplex Virus Type 1 Infection by ND10 Components ATRX and hDaxx. *J Virol* 84(8):4026–4040

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.