Journal of Plant Interactions

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/tjpi20

Study of interaction between *Papaya ringspot virus* coat protein and infected *Carica papaya* proteins

Wanwisa Siriwan, Sittiruk Roytrakul, Srimek Chowpongpang & Aroonothai Sawwa

To cite this article: Wanwisa Siriwan, Sittiruk Roytrakul, Srimek Chowpongpang & Aroonothai Sawwa (2021) Study of interaction between *Papaya ringspot virus* coat protein and infected *Carica papaya* proteins, Journal of Plant Interactions, 16:1, 474-480, DOI: 10.1080/17429145.2021.1981470

To link to this article: https://doi.org/10.1080/17429145.2021.1981470

Published online: 04 Oct 2021.

JOURNAL OF PLANT INTERACTIONS
2021, VOL. 16, NO. 1, 474–480
https://doi.org/10.1080/17429145.2021.1981470

Taylor & Francis
Taylor & Francis Group

PLANT-MICROORGANISM INTERACTIONS

Study of interaction between *Papaya ringspot virus* coat protein and infected *Carica papaya* proteins

Wanwisa Siriwan [a,b,c], Sittiruk Roytrakul [d], Srimek Chowpongpang[e] and Aroonothai Sawwa[f]

[a]Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand; [b]Center for Agricultural Biotechnology, Kasetsart University, Nakhon Pathom, Thailand; [c]Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok, Thailand; [d]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (NSTDA), Pathumtani, Thailand; [e]National Science and Technology Development Agency, Pathum Thani, Thailand; [f]Department of Agricultural, Biotechnology Research and Development Office, Pathum Thani, Thailand

ABSTRACT

Papaya ringspot virus (PRSV) causes the Papaya ringspot disease. Virus–host interactions appear to play a significant role in the replication, pathogenesis, and infection caused by the PRSV. PRSV coat protein is likely to be involved in the processes of RNA replication, aphid transmission, and cell-to-cell movement, which is closely related to the host cell proteins resulting in a PRSV infection. To identify the host proteins that interact with the CP in vitro, immune precipitation, in-solution trypsin digestion, and LC–MS/MS were performed. Twenty-three identified proteins that interacted with the CP are involved in cellular metabolism, transcription, signal, translation, carbohydrate metabolism, protein metabolism, stress response, photosynthesis, nucleotide metabolism, respiration, and lipid metabolism processes. The search tool for interactions of chemicals (STITCH) results show that SWP (an RNA polymerase II transcription mediator) and MPPBETA (Mitochondrial processing peptidase) are involved in known plant defense mechanisms including transcription factors, cell division, hormones, stress, mitochondrial electron transferase, respiration, and proteasome. Therefore, analyzing virus–host protein interactions at a molecular level is important to build a better understanding of the virus replication mechanism and cellular responses mounted against viruses by the host defense system.

ARTICLE HISTORY
Received 1 February 2021
Accepted 13 September 2021

KEYWORDS
Protein–protein interaction; *Papaya ringspot virus*; coat protein; affinity purification mass spectrometry; LC–MS/MS and STITCH analysis

Introduction

Papaya ringspot disease caused by the *Papaya ringspot virus* (PRSV), which is a member of the *Potyviridea* genus. *Potyvirus* is recognized as the largest genus by the International Committee on Taxonomy of Viruses (ICTV 2020). More than 200 species of viruses have been reported to cause significant crop losses around the world Shukla et al. (1994). *Potato virus Y* (PVY), *papaya ring spot virus* (PRSV), *sugarcane mosaic virus* (SCMV), *pepper mottle virus* (PepMoV), and *zucchini yellow mosaic virus* (ZYMV) are the most serious disease-causing viruses in Thailand (Bateson et al. 2002; Gemechu et al. 2006). Thailand's Papaya production is also hampered by the papaya ringspot disease, affecting farmer income, healthy papaya production, as well as crop sustainability. More than 200 species of aphid are virus vectors (subfamily *Aphidinae*).

PRSV is the main pathogen affecting the papaya crop (*Carica papaya* L.). The PRSV translated protein is a polyprotein, cleaved by three proteinases and separated into ten proteins, including a genome-linked protein (VPg), P1 serine proteinase (P1), helper component-protein (HC-Pro), P3 protein, 6K1 protein, cylindrical inclusion protein (CI), 6K2 protein, nuclear inclusion protein (NIa), large nuclear inclusion protein (NIb), and coat protein (CP) (Shukla et al. 1994). CP is an essential protein for the survival of a virus and responsible for various viral infection mechanisms and steps, the study of the interaction between CP and papaya proteins has gained attraction amongst the community. Moreover, CP is also a multifunctional protein and is involved in the intercellular movement (Dolja et al. 1995), virus assembly (Jagadish et al. 1991; Varrelmann and Maiss 2020), aphid transmission (Atreya et al. 1990; Shukla et al. 1994; Silva-Rosales et al. 2000), and plasmodesmatal gating (Rojas et al. 1997). CP structure and functions have been known since the earliest PRSV outbreaks in the West and Asia. Seo et al. (2010) have demonstrated that the potyvirus CP interacts with viral proteins such as HC-Pro protein, affecting virus accumulation, aphid transmission, and long-distance movement of the *soybean mosaic virus*.

It is important to study the protein–protein interactions, as they form a vital step in identifying the functions of a viral protein and the interaction mechanism between the virus and host cells. Currently, the interaction networks between virus and host are still poorly understood due to a lack of research data in this field. Yeast two-hybrid system (YTHS) is an extensive tool to study such protein–protein interactions and is a well-established classical biochemical and genetic *in vivo* approach. More than 5600 protein interactions have been reported using this method, including the establishment of general protein interaction networks (Uetz and Hughes 2000; Uetz et al. 2000; Ito et al. 2001). However, some groups of protein–protein interactions can be lost in YTHS. For example, the interactions related to membrane proteins, self-activating proteins, and post-translational protein modifications (Brückner et al. 2009). Another method also used to screen protein–protein interactions is

CONTACT Wanwisa Siriwan wanwisa.si@ku.th Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand

affinity purification mass spectrometry (AP-MS). AP-MS has become a highly efficient tool to discover protein–protein interactions in various creatures and to identify the dynamics of protein–protein interactions in *in vivo* analysis to probe the protein interactions under certain conditions. This approach can capture higher-order components of a larger complex, with the information being crucial to determine the function of a protein in plant cellular (Zhang et al. 2010; Morris et al. 2014). Previously, we analyzed the protein–protein interactions between 23 papaya (*Carica papaya*) proteins and PRSV HC–Pro protein using the AP-MS approach (Siriwan et al. 2014). Recently, AP-MS has also been applied to explore the interaction of non-structure protein 1 of the dengue virus with the host cell proteins (Dechtawewat et al. 2016).

In this study, we report on the PRSV CP interactions with the plant's proteins of infected papaya plants. Furthermore, a similar immune precipitation method in cooperated with an in-solution trypsin digestion and liquid chromatography–tandem mass spectrometer was also performed. The study of the interaction between CP and papaya proteins will help us elucidate the molecular mechanisms underling the relationship between viruses and hosts as well as to establish control and management measures to prevent further disease spread.

Materials and methods

Plant preparation and inoculation

Papaya plants were cultivated under controlled conditions in a greenhouse maintained at 24°C, with an 8 h exposure to light and 16 h in dark at 60% humidity. The plants were inoculated when they had more than six leaves. One gram of PRSV-infected papaya leaves was powdered using liquid nitrogen (N_2), and subsequently, 5 ml of 0.1 M phosphate buffer saline (PBS) at a pH 7.4 was added. The carborundum powders were mixed with the PRSV-infected sap and the mixture was rubbed on the leaf surface to facilitate the delivery of the virus into the plant, which was left for 10 min and washed with water. The mock control was Phosphate buffered saline (PBS) buffer at a pH 7.4 mixed with carborundum powders rubbed on the leaf surface. The symptoms appeared within 7–10 days after inoculation. In the next step, infected leaf samples were collected for protein extraction.

Construction of PRSV CP recombinant protein

The RNA from PRSV-infected plants was extracted using the RNeasy Plant Mini RNA extraction kit method (Qiagen, Cincinnati, OH, USA). The cDNA synthesis was actualized by using M-MLV (Invitrogen, Carlsbad, CA, USA), according to the standard instructions of the manufacturer. One microgram of the total RNA was reverse-transcribed with 10 µM of Oligo-dT primer, 1 µl dNTPs (each 2.5 mM), and sterile distilled water, to obtain a final volume of 20 µl. After incubation at 65°C for 5 min, RNase inhibitor, 0.1 M Dichlorodiphenyltrichloroethane (DTT), 5× buffer solution, and 200 U of M-MLV enzymes were added. This mixture was then incubated at 37°C for 150 min. The PCR reaction mixture contained 20 ng of first stand cDNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 pmole primers (CP_Forward; 5′GCG GAT CCT CC A AAA CTG AAG CTG TGG ATG CTG G 3′ and CP_Reverse; 5′GCG CGG CCG CTT AAG ACT TGA CAT TGC GCA TAC CCA GGA GAG AGT 3′), 0.5 U *Taq* enzyme (Promega, Medison, WI, USA) in a final total volume of 20 µl. Thirty five PCR cycles at 95°C for 1 min, at 60°C for 1 min, and at 72°C for 1 min were performed. PCR products were separated by 1.5% (w/v) agarose gels electrophoresis in a 1×TBE buffer and visualized under UV light after straining with ethidium bromide.

The PCR products were digested with BamHI and NotI restriction enzymes and ligated into pET28a (+) (Novagen, Madison, WI, USA) to generate recombinant plasmids 6xHisCPpET28a(+) in *Escherichia coli* DH5α. The PRSV-CP gene fragment was 1,070 bp. The reading frames of inserted genes were confirmed by a DNA sequence analysis (Capillary Electrophoresis on the ABI Prism 310) and Soft-Berry-Alignments (Yu et al. 2002)

Expression of recombinant PRSV-CP

The PRSV-CP fragment was inserted into the pET28a(+) expression vector and transformed into *E.coli* JM109 (DE3). The recombinant colony was cultured in 50 ml of Luria–Bertani medium (LB) with Kanamycin (50 µg/mL) at a temperature of 30°C by shaking at 120 rpm. A starter culture (OD_{600} 0.55–0.6) was added into 5,000 mL of fresh LB with Kanamycin (50 µg/mL), and the shaking was continued at 120 rpm, maintained at a temperature of 30°C. The recombinant protein was induced by adding isopropyl-β-D-thiogalactopyranoside (IPTG) at a final concentration of 0.8 mM and when the OD_{600} culture had reached 0.6. After gentle shaking for 6 h, bacterial cells were then collected by centrifugation (5500 rpm for 10 min at 4°C). The cell pellet was collected, re-suspended in 5 ml of binding buffer (made of 20 mM, sodium phosphate, 500 mM NaCl, 10 mM imidazole at a pH of 7.4, and 5 mM DTT) and sonicated for 15 min. The soluble protein fraction was loaded onto a 1 ml HisTrap FF crude column (GE Healthcare, Pascataway, NJ, USA), which was connected to a Fast Flow Liquid Chromatograph (FPLC, GE Healthcare, Pascataway, NJ, USA). The recombinant protein was eluted with 0.8 M Imidazole. The recombinant PRSV CP fraction was separated by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and stained with 0.2% (w/v) Coomassie blue R250. The purified protein concentration was measured by using Bio-Rad Protein Assay (Bio-RAD, Hercules, CA, USA), which is based on the Bradford method. The purified recombinant proteins were used in pull down protein assay.

Total papaya protein extraction

The total proteins were extracted from PRSV infected papaya leaves with a slightly modified form of Trichloroacetic acid Trichloroacetic Acid (TCA)-acetone precipitation procedure (Rodrigues et al. 2009). Fifteen grams of PRSV infected leaves sample was homogenized in liquid nitrogen (N_2) and the total proteins were precipitated in 15 ml acetone, 10% (v/v) TCA, and 0.07% (v/v) β-mercaptoethanol overnight at 20°C. The supernatant was discarded after centrifugation at 8000 rpm for 30 min at a temperature of 4°C. Next, the protein pellets were washed three times in cold acetone pulsed 0.07% (v/v) β-mercaptoethanol and dried by a

speed vacuum concentrator. The dried pellets were re-suspended in 2 ml of resolubilisation buffer (7 M urea, 2 M thiourea, 4% (w/v) 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 1% (w/v) DTT), and sonicated for 30 min at 4°C and vortexed for 10 min. The supernatant was collected after centrifugation at 10,000 rpm for 30 min at 4°C. The total protein concentration was measured using a Bio-Rad Protein Assay (Bio-RAD, Hercules, CA, USA).

In vitro pull-down assay

The pull-down assay composition comprised of a recombinant CP (bait) and total plant protein (prey). Two milliliter of nickel beads were prewashed with H_2O and a native binding buffer (ProBond nickel-chelating resin beads, Invitrogen). The experiments were performed on three replicates. The mixtures included 0.25 mg/ml of bait and pray protein each and the nickel beads were incubated at 7°C and gently rolled for 2 h. After washing thrice with a native washing buffer, the beads were collected. The bait-prey protein complex was eluted with a native elution buffer (250 mM imidazole in a native binding buffer) and collected for the LC–MS/MS analysis.

Liquid chromatography–tandem MS (LC–MS/MS) analysis

The interacting protein complex fractions obtained from the in vitro pull-down assay were directly applied to ZebaTM Spin Desalting columns (Thermo Scientific Inc, Beverly, MA, USA) and the protein concentration in each column was desalted and determined by Lowry assay using BSA as the standard protein. During the solution digestion, each fraction was hydrolyzed by trypsin at an enzyme to protein ratio of 1:50 and incubated at 37°C for 24 h. The peptides were then dried in a vacuum centrifuge. The extracted peptides were dissolved in 15 µl of 0.1% (v/v) formic aid, centrifuged at 10,000 rpm for 10 min and separated using an Ultimate 3000 LC System (Dionex Ltd., Leeds, UK) on a nanocolumn PepSwift monolithic column 100 mm i.d.650 mm, with a flow rate of 300 nl/min using a multi-step gradient of linear concentration increase from 10% to 90% of 80% (v/v) acetonitrile in 0.1% (v/v) formic acid within a duration of 20 min. The nanoLC system was connected with an electrospray interface with an ESI-Ion Trap MS (Bruker Daltonik GmbH, Bremen, Germany). The collected LC–MS raw data were directly used for the quantification based on the individual MS signal intensities determined by the DeCyder MS differential analysis software (DeCyderMS, GE Healthcare). All the differential peptide data were searched against the NCBI *Arabidopsis thaliana* database using the MASCOT software version 2.2 (Matrix Science, London, UK). The search parameters used were as follows: trypsin was selected as the enzyme, with three potential missed cleavages, carbamido-methylated cysteine as a fixed modification, and oxidation of methionine residues as a variable modification. Peptide mass tolerance was kept at 1.2 Da, while fragment mass tolerance was kept at 0.6 Da, and the ESI ion trap was selected as the instrument type. Proteins were selected with a statistical significance of $P < .05$ as shown in the maps of protein levels using the Multi Experiment Viewer (MeV, version 4.6.1) software.

Bioinformatics analysis

The biological interpretation and function of protein identification were analyzed through GeneOntology (Go) Cat (http://eagl.unige.ch/GOCat/) and Uniprot (http://www.uniprot.org) sequence databases. Protein–protein interaction was determined according to the search tool for interactions of chemicals (STITCH) 4.0 database (http://stitch.embl.de/). STITCH is a database containing protein–chemical interactions and includes predicted interactions based on text mining and other prediction methods.

Results

The recombinant PRSV CP

PRSV was inoculated into the papaya plants on the first and second leaves of healthy papaya plants. The mosaic symptoms presented at day 7 after inoculation. The inoculated PRSV plants were diagnosed by RT–PCR with primers specific for CP. RT–PCR result of PRSV infected sample indicated to 450-bp DNA fragment which was similar to positive control. The DNA sequence of amplicon resulted in a 100% matched with PRSV CP.

To construct a recombinant CP, amplified CP fragments were ligated into pET28a(+) and named 6xHisCPpET28a(+). The recombinant protein plasmids were transformed to *E. coli* strain JM109 (DE3) and then cell cultures were induced by 0.8 M IPTG for 6 hrs to produce the proteins. The result of SDS-PAGE analysis demonstrated that the recombinant coat protein yielded a 36 kDa soluble protein.

Pull down assay and LC–MS/MS analysis

The pull-down protein complexes were identified by LC–MS/MS analysis. Two samples of protein complexes from an infected and non-infected plant interacted with CP were digested in-solution through the trypsin enzyme. Three replications were obtained for each sample. The peptide samples were subsequently processed and labeled through the free shotgun proteomic analysis by LC–MS/MS. The DeCyder MS differential analysis software was used to align and quantify the raw MS data. The MASCOT search engine was used to identify the differentially expressed proteins against the green plant proteins database. The peptide ion intensities of these proteins were normalized against each control sample and any proteins with an average ratio above 1.5 were selected from the overall data. Through the criteria listed above, 33 proteins were identified as potential host proteins that could have interacted with the PRSV CP. However, 12 plant proteins were unnamed and hypothetical proteins. The identified proteins are listed in Table 1. The biological functions of the identified proteins were classified into 10 groups which consist of transcription (22%), signal (17%), translation (13%), carbohydrate metabolism (9%), protein metabolism (9%), stress response (9%), photosynthesis (9%), nucleotide metabolism (4%), respiration (4%), and lipid metabolism (4%) (Figure 1).

The possible functional interactions of the identified proteins were predicted using STITCH 5 software based on the following analysis parameters: species (*Arabidopsis thaliana*); medium confidence score (0.04); and active prediction methods (all and no more than 10). RNA polymerase II transcription mediator (SWP) and mitochondrial processing peptidase (MPPBETA) were predicted to determine cell

Table 1. PRSV coat protein interacting host proteins identified in PRSV infected papaya plant.

No.	Protein name	GI number	UniProt accession number	Peptides	Intensity ratio of PRSV-CP to PRSV infected plant	Intensity ratio of PRSV-CP to Native buffer
1	Beta galactosidase 1	gi|15231354	Q9SCW1	GHAMH	118.14	0
2	Calmodulin-domain protein kinase (CDPK)	gi|15228350	Q38870	IMGGPV	122.72	0
3	Carbamoyl-phosphate synthase	gi|255075411	C1E3Y9	GVTPSMK	122.72	0
4	Cysteine synthase like protein	gi|41351505	Q767A2	PGNPKTH	122.56	0
5	Doc	gi|46401725	Q06259	GLPGMSDPG	100.67	0
6	E2 polyprotein	gi|151367859	A7KYB7	VSEVLGGAGLPG	100.67	0
7	Envelope glycoprotein	gi|154361337	A7L258	AXGBI	119.74	0
8	GIF1	gi|293651114	D5L5T9	DAAXXX	100.67	0
9	Glycosylphosphatidylinositol anchor attachment protein GAA1 (ISS)	gi|308807647	-	SGDPAGHRS	100.67	0
10	Heat shock protein 101	gi|82408817	Q2VDS9	KAHPA	100.67	0
11	Hydroxyphenylpyruvate dioxygenase	gi|154240639	A7BG64	AVGNVPN	118.91	0
12	LHC II Type III chlorophyll a/b binding protein	gi|405615	Q39340	SSSFNP	100.67	0
13	Mitochondrial processing peptidase	gi|15232845	Q42290	AIGPI	119.20	0
14	Myb-like protein	gi|27261073	Q8GVH9	TGGSANGMD	118.22	0
15	NADH dehydrogenase, partial (chloroplast)	gi|12656882	O46939	KXXGP	119.52	0
16	Nephrocystin-4-like protein	gi|159469538	A8IVX5	VVRGAVS	118.50	0
17	Pentatricopeptide repeat-containing protein	gi|240254458	Q9XIL5	KVGDFAKA	100.67	0
18	Putative lipoxygenase	gi|27436755	Q8H016	THIAG	120.96	0
19	Ribosomal protein L12 homolog	gi|2331135	O22386	ARIAS	100.67	0
20	Ribosomal protein S2	gi|11465943	P06355	DAISS	118.31	0
21	Ribulose-1,5-bisphosphate carboxylase	gi|10179652	Q9GE62	XPGGX	119.41	0
22	RNA polymerase II transcription mediator	gi|15229344	Q9SR02	DGSGKP	117.09	0
23	RNA-dependent RNA-polymerase	gi|57545936	Q3ZN03	AAFMA	117.92	0

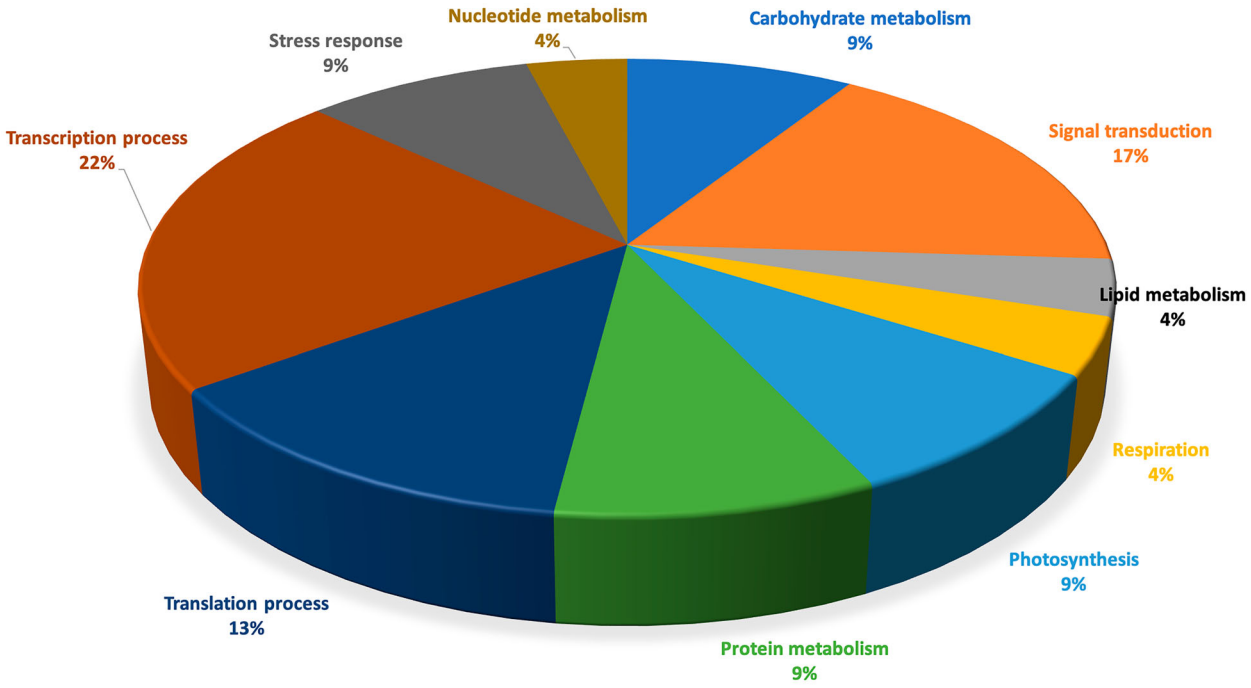

Figure 1. Classification of PRSV coat protein-interacting with PRSV infected papaya proteins. Twenty-three identified PRSV coat protein-interacting host cell proteins were mapped to the UniProt database. Illustrate the molecular functions and biological processes of these PRSV coat protein-interacting host cell proteins. Ten categories among the total twenty-three interacted protein are transcription, signallng, translation, carbohydrate metabolism, protein metabolism, stress response, photosynthesis, nucleotide metabolism, respiration and lipid metabolism.

division, transcription factors, electron transferase, respiration, proteasome subunit, translation initiation, hormones, and stress. On the other hand, Calmodulin-domain protein kinase (CPK2), Pentatricopeptide repeat-containing protein (OTP51), and Beta galactosidase 1 (BGAL) are presented in the STITCH diagram, but they were not interact with those proteins (Figure 2).

Discussion

The interplay between viral and host proteins is particularly important to understand the mechanisms and functions of the PRSV infection and host cellular response. The functions of most interacting proteins were classified and were related to signaling, transcription, and translation processes. Some interacting protein partners were involved in stress response and plant physiology. However, some interacting proteins produced results similar to those previously reported, such as in *alfalfa mosaic virus*, with the CP binding to 3′-terminal RNA-dependent RNA-polymerase (RdRp) at the initiation site resulting in an in vitro antisense strand synthesis (Reichert et al. 2007). In addition, RdRp was reported to be induced by viruses, which belong to post-transcriptional gene silencing (PTGS) (Ratcliff et al. 1997; Al-Kaff et al. 1998). Xie

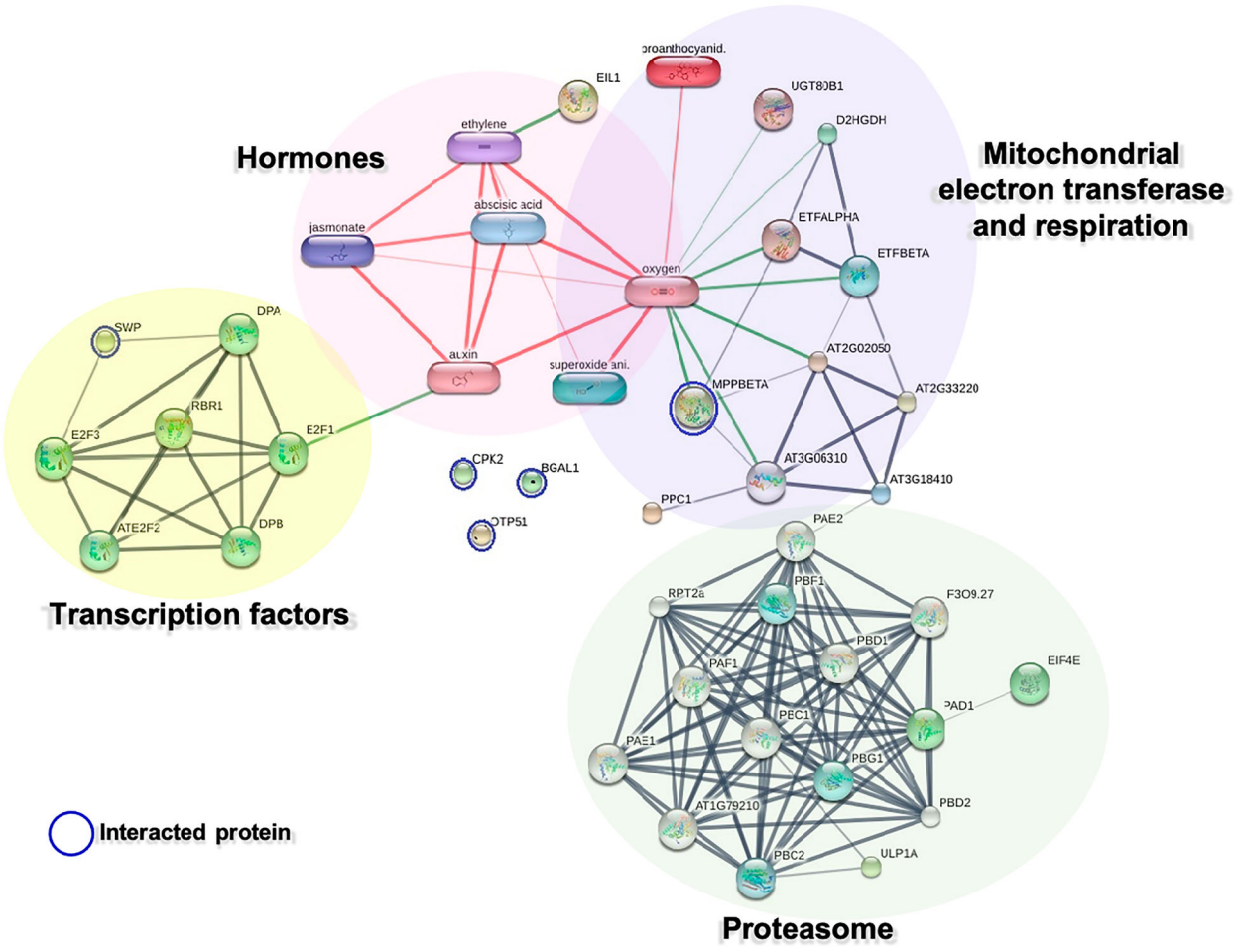

Figure 2. The involvement of 23 candidate proteins in the intrinsic and extrinsic cellular pathway predicted by STITCH. Based on the online STITCH 4.0 database, an association of 23 candidate proteins. SWP (RNA polymerase II transcription mediator) and MPPBETA (Mitochondrial processing peptidase) are involve were predicted to involve in the biological processes. CPK2 (Calmodulin-domain protein kinase), OTP51 (Pentatricopeptide repeat-containing protein) and BGAL1 interacted candidates protein do not play role in the interaction map. Modes of action are shown in different color lines. Blue circles indicate identified proteins in this study.

et al. (2001) found that the activity of a tobacco RdRp was increased at the time of virus infection. This indicated that RdRp played an important role in the infection cycle or for suppression of immunity defense, while being targeted by antiviral responses (Shen et al. 2020). It also indicated that CP *Potato virus X* (PVX) activated a membrane bound calcium/calmodulin dependent kinase (CDPK), in addition to playing a key role in the activation of an early defense response *Arabidopsis thaliana*, which was related to phosphorylation targets in abiotic stress signaling (Perraki et al. 2017).

To quantify the correlation between the identified proteins, we used the STITCH (Search Tool for Interacting Chemicals) network program to predict the protein interaction networks as well as the interacting proteins and small molecules. The STITCH results showed four large groups of interacting proteins were related to each other. The four groups were divided into group 1: transcription factors and cell division; group 2: hormones and stress; group 3: mitochondrial electron transferase and respiration; and group 4: proteasome. We found that 5 out of 23 interacting proteins were detected in the STITCH network. SWP (RNA polymerase II transcription mediator) and MPPBETA (Mitochondrial processing peptidase) are involved in all these four groups. SWP is a component of the Mediator complex, a co-activator involved in the regulating transcription of nearly all RNA polymerase II-dependent genes. This Mediator functions as a bridge to convey information from gene-specific regulatory proteins to the basal RNA polymerase II transcription machinery. The Mediator complex, having a compact conformation in its free form, is recruited to promote direct interactions with regulatory proteins and serves in the assembly of a functional preinitiation complex with RNA polymerase II. MPPBETA, whose probable function was identified as mitochondrial processing of peptidase subunit beta, mitochondrial; cleaving pre-sequences (transit peptides) from mitochondrial protein precursors, belongs to the peptidase M16 family.

Interestingly, we found that CPK2, OTP51 (Pentatricopeptide repeat-containing protein), and BGAL1 were not linked with any known plant defense mechanisms including transcription factors, cell division, hormones, stress, mitochondrial electron transferase, respiration, and proteasome. PRSV may need other proteins or small molecules to enter a host cell that could identify those molecules in the future. CPK2 encodes calcium-dependent protein kinase and is localized in the endoplasmic reticulum. OTP51 function encodes a protein that promotes splicing of type II introns. OTP51 may be involved in splicing several other transcripts and precursor forms of the trnL, trnG, trnI, and trnA transcripts. However, the predicted function of BGAL1 was not apparent in the STITCH analysis.

E2 polyprotein, envelop glycoprotein, NADH dehydrogenase, and RNA polymerase II transcription mediator interacting proteins were predicted to mediate in the plant-viral interaction and host immune response. Interestingly, RDRs (a member of the RNA polymerase II transcription Mediator) was found to play an important role in a plant's antiviral defense mechanism via silencing-mediated regulation of the cellular mRNA encoding resistance factors. On the other hand, the level of RDR expression was induced by salicylic acid (SA) and jasmonic acid (JA) in *Nicotiana attenuate* when challenged by the TMV. However, the RDR gene regulation is still not well understood (Xie et al. 2001; Yu et al. 2003; Schwach et al. 2005; Wassenegger and Krczal 2006; Qin et al. 2017).

Viral infection damages a plant's physiological systems. A consequence of deformed pathway shows up as mosaic or yellowing in plants. The infection interferes with the process of photosynthesis. A range of symptoms can be exhibited, based on whether or not the host–virus combination is incompatible or compatible (Almasi et al. 2001). In our study, the PRSV CP interacted with LHC II type III chlorophyl a/b binding protein and ribulose-1, 5-bisphosphate carboxylase. It is a major protein involved in photosynthesis and is located in the cytoplasm and chloroplast. Dietzgen and Zaitlin (1986) reported that the function of TMV coat protein was to reduce the amount of the most abundant host proteins-ribulose bisphosphate carboxylase-oxygenase (rbcs or rubisco) and is one of the most common damages caused by viruses which causes mosaic and yellowing disease. In tobacco plants, *cucumber mosaic virus* (CMV) variants containing different CP mutations result in chlorosis symptoms at varying levels of severity, which is correlated with down regulation of photosynthesis-related genes (CPRGs) (Mochizuki et al. 2014).

Our findings confirm that networks of coat protein of potyvirus and host protein interaction are highly connected, with some proteins playing the role of hubs. However, interacted proteins results of this study did not directly connect with HC-Pro interacted protein that Siriwan et al. (2014) reported. Bosque et al. (2014) represented HC-Pro protein might interact with CP through CI, VPg and NIaPro or intermediary plant proteins. It might require a systems approach such as BiFC method and the pool of reliable intraviral interaction tested and detected increases and topology analysis to be fully described on data set of this study and previously reported. More detail and systematic understanding of how viral protein interact with each other, and with host protein might allow to developing new drugs and treatment that block viral replication.

With regards to a simplistic plant pathological and plant management viewpoint, functional proteomics via viral and plant protein interaction may help to understand how a virus enters a plant cell and how the plant mounts a defense. Additionally, investigating plant virus infectious cycles and host-potyvirus interactions is essential for the development of anti-viral strategies and resistant cultivars.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research was supported by the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Office of Higher Education Commission, Ministry of Education (AG-BIO/PERDO-CHE).

Notes on contributors

Wanwisa Siriwan is lecturer working in Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Thailand.

Sittiruk Roytrakul is senior researcher working in the National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (NSTDA), Thailand Science Park, Thailand.

Srimek Chowpongpang is senior researcher working in the National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (NSTDA), Thailand Science Park, Thailand.

Aroonothai Sawwa is senior agriculture scientist working in Biotechnology Research and Development Office, Department of Agricultural, Thailand.

ORCID

Wanwisa Siriwan http://orcid.org/0000-0002-5603-7844
Sittiruk Roytrakul http://orcid.org/0000-0003-3696-8390

References

Al-Kaff NS, Covey SN, Kreike MM, Page AM, Pinder R, Dale PJ. 1998. Transcriptional and posttranscriptional plant gene silencing in response to a pathogen. Science. 279(5359):2113–2115. doi:10.1126/science.279.5359.2113.

Almási A, Harsányi A, Gáborjányi R. 2001. Photosynthetic alterations of virus infected plants. Acta Phytopathologica et Entomologica Hungarica. 36:15–29.

Atreya CD, Raccah B, Pirone TP. 1990. A point mutation in the coat protein abolishes aphid transmissibility of a potyvirus. Virology. 178:161–165.

Bateson MF, Lines RE, Revill P, Chaleeprom W, Ha CV, Gibbs AJ, Dale JL. 2002. On the evolution and molecular epidemiology of the potyvirus *Papaya ringspot virus*. J Gen Virol. 83(10):2575–2585. http://vir.sgmjournals.org/cgi/content/abstract/83/10/2575.

Bosque G, Folch-Fortuny A, Picó J, Ferrer A, Elena SF. 2014. Topology analysis and visualization of potyvirus protein-protein interaction network. BMC Syst Biol. 8(1):129. doi:10.1186/s12918-014-0129-8.

Brückner A, Polge C, Lentze N, Auerbach D, Schlattner U. 2009. Yeast two-hybrid, a powerful tool for systems biology. Int J Mol Sci. 10 (6):2763–2788. doi:10.3390/ijms10062763.

Dechtawewat T, Paemanee A, Roytrakul S, Songprakhon P, Limjindaporn T, Yenchitsomanus P-T, Saitornuang S, Puttikhunt C, Kasinrerk W, Malasit P. 2016. Mass spectrometric analysis of host cell proteins interacting with dengue virus nonstructural protein 1 in dengue virus-infected HepG2 cells. Biochimica et Biophysica Acta (BBA) – Proteins and Proteomics. 1864(9):1270–1280. doi:10.1016/j.bbapap.2016.04.008.

Dietzgen RG, Zaitlin M. 1986. Tobacco mosaic virus coat protein and the large subunit of the host protein ribulose-1,5-biphosphate carboxylase share a common antigenic determinant. Virology. 155:262–266.

Dolja VV, Haldeman-Cahill R, Montgomery AE, Vandenbosch KA, Carrington JC. 1995. Capsid protein determinants involved in cell-to-cell and long distance movement of tobacco etch potyvirus. Virology. 206:1007–1016.

Gemechu AL, Chiemsombat P, Attathom S, Reanwarakorn K, Lersrutaiyotin R. 2006. Cloning and sequence analysis of coat protein gene for characterization of sugarcane mosaic virus isolated from sugarcane and maize in Thailand. Arch Virol. 151(1):167–172. doi:10.1007/s00705-005-0643-5.

ICTV. 2020. Potyvirus. [accessed 2021 Jan 21]. https://talk.ictvonline.org/taxonomy/.

Ito T, Chiba T, Ozawa R, Yoshida M, Hattori M, Sakaki Y. 2001. A comprehensive two-hybrid analysis to explore the yeast protein interactome. Proc Natl Acad Sci USA. 98(8):4569–4574. doi:10.1073/pnas.061034498.

Jagadish M, Ward CW, Gough KH, Tulloch PA, Whittaker LA, Shukla DD. 1991. Expression of potyvirus coat protein in *Escherichia coli* and yeast and its assembly into virus-like particles. J Gen Virol. 72:1543–1550.

Mochizuki T, Ogata Y, Hirata Y, Ohki ST. 2014. Quantitative transcriptional changes associated with chlorosis severity in mosaic leaves of tobacco plants infected with cucumber mosaic virus. Mol Plant Pathol. 15(3):242–254. doi:10.1111/mpp.12081.

Morris JH, Knudsen GM, Verschueren E, Johnson JR, Cimermancic P, Greninger AL, Pico AR. 2014. Affinity purification–mass spectrometry and network analysis to understand protein-protein interactions. Nat Protoc. 9:2539. doi:10.1038/nprot.2014.164 https://www.nature.com/articles/nprot.2014.164#supplementary-information

Perraki A, Gronnier J, Gouguet P, Boudsocq M, Deroubaix A-F, Simon V, German-Retana S, Zipfel C, Bayer E, Mongrand S, et al. 2017. The plant calcium-dependent protein kinase CPK3 phosphorylates REM1.3 to restrict viral infection. bioRxiv. doi:10.1101/205765.

Qin L, Mo N, Zhang Y, Muhammad T, Zhao G, Zhang Y, Liang Y. 2017. CaRDR1, an RNA-dependent RNA polymerase plays a positive role in pepper resistance against TMV. Front Plant Sci. 8:1068–1068. doi:10.3389/fpls.2017.01068.

Ratcliff F, Harrison BD, Baulcombe DC. 1997. A similarity between viral defense and gene silencing in plants. Science. 276 (5318):1558–1560. doi:10.1126/science.276.5318.1558.

Reichert VL, Choi M, Petrillo JE, Gehrke L. 2007. Alfalfa mosaic virus coat protein bridges RNA and RNA-dependent RNA polymerase in vitro. Virology. 364(1):214–226. doi:10.1016/j.virol.2007.02.026.

Rodrigues SP, Ventura JA, Zingali RB, Fernandes PMB. 2009. Evaluation of sample preparation methods for the analysis of papaya leaf proteins through two-dimensional gel electrophoresis. Phytochem Anal. 20(6):456–464. doi:10.1002/pca.1147.

Rojas MR, Zerbini FM, Allison RF, Gilbertson RL, Lucas WJ. 1997. Capsid protein and helper component-proteinase function as potyvirus cell-to-cell movement proteins. Virology. 237:283–295.

Schwach F, Vaistij FE, Jones L, Baulcombe DC. 2005. An RNA-dependent RNA polymerase prevents meristem invasion by *Potato virus* X and is required for the activity but not the production of a systemic silencing signal. Plant Physiol. 138(4):1842–1852. doi:10.1104/pp.105.063537.

Seo J-K, Kang S-H, Seo BY, Jung JK, Kim K-H. 2010. Mutational analysis of interaction between coat protein and helper component-proteinase of soybean mosaic virus involved in aphid transmission. Mol Plant Pathol. 11(2):265–276. doi:10.1111/j.1364-3703.2009.00603.x.

Shen W, Shi Y, Dai Z, Wang A. 2020. The RNA-dependent RNA polymerase NIb of potyviruses Plays multifunctional, contrasting roles during viral infection. Viruses. 12:1. doi:10.3390/v12010077.

Shukla DD, Ward CW, Brunt AA. 1994. *The potyviridae*. Wallingford: CAB International; 516 pp.

Silva-Rosales L, Becerra-Leor N, Ruiz-Castro S, Téliz-Ortiz D, Noa-Carrazana JC. 2000. Coat protein sequence comparisons of three Medican isolates of papaya ringspot virus with other geographical isolates reveal a close relationship to American and Australian isolates. Arch Virol. 145:835–843.

Siriwan W, Takaya N, Roytrakul S, Chowpongpang S. 2014. Study of interaction between papaya ringspot virus HC-Pro and papaya (*Carica papaya*) proteins. J Gen Plant Pathol. 80(3):264–271. doi:10.1007/s10327-014-0523-5.

Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, Knight JR, Lockshon D, Narayan V, Srinivasan M, Pochart P, et al. 2000. A comprehensive analysis of protein–protein interactions in *Saccharomyces cerevisiae*. Nature. 403:623. doi:10.1038/35001009.

Uetz P, Hughes RE. 2000. Systematic and large-scale two-hybrid screens. Curr Opin Microbiol. 3(3):303–308. doi:10.1016/S1369-5274(00)00094-1.

Varrelmann M, Maiss E. 2020. Mutations in the coat protein gene of plum pox virus suppress particle assembly, heterologous encapsidation and complementation in transgenic plants of *Nicotiana benthamiana*. J Gen Virol. 81:567–576.

Wassenegger M, Krczal G. 2006. Nomenclature and functions of RNA-directed RNA polymerases. Trends Plant Sci. 11(3):142–151. doi:10.1016/j.tplants.2006.01.003.

Xie Z, Fan B, Chen C, Chen Z. 2001. An important role of an inducible RNA-dependent RNA polymerase in plant antiviral defense. Proc Natl Acad Sci USA. 98(11):6516–6521. doi:10.1073/pnas.111440998.

Yu D, Fan B, MacFarlane SA, Chen Z. 2003. Analysis of the involvement of an inducible Arabidopsis RNA-dependent RNA polymerase in antiviral defense. Mol Plant Microbe Interact. 16(3):206–216. doi:10.1094/mpmi.2003.16.3.206.

Yu J, Hu S, Wang J, Wong GK-S, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, et al. 2002. A draft sequence of the rice genome (*Oryza sativa* L. ssp. indica). Science. 296(5565):79–92. doi:10.1126/science.1068037%J.

Zhang Y, Gao P, Yuan JS. 2010. Plant protein-protein interaction network and interactome. Curr Genomics. 11(1):40–46. doi:10.2174/138920210790218016.