Applied Science and Engineering Progress, Vol. 13, No. 3, pp. 224–232, 2020

Research Article

Identification of GST Interacted Proteins under PRSV Infected Papaya Using Affinity Purification–mass Spectrometry

Aroonothai Sawwa
Biotechnology Research and Development Office, Department of Agricultural, Rangsit, Pathum Thani, Thailand
Center for Agricultural Biotechnology, Kasetsart University Kampaeng Saen Campus, Nakhorn Pathom, Thailand
Center of Excellence on Agricultural Biotechnology: AG-BIO/PERDO-CHE), Bangkok, Thailand

Yodying Yingchutrakul and Sittiruk Roytrakul
National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Pathum Thani, Thailand

Wanwisa Siriwan
Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand

Srimek Chowpongpang*
Center for Agricultural Biotechnology, Kasetsart University Kampaeng Saen Campus, Nakhorn Pathom, Thailand
Center of Excellence on Agricultural Biotechnology: AG-BIO/PERDO-CHE), Bangkok, Thailand
Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand
National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Pathum Thani, Thailand

* Corresponding author. E-mail: aroonothai.s@doa.in.th DOI: 10.14416/j.ijast.2019.01.006
Received: 28 June 2018; Revised 10 August 2018; Accepted: 15 August 2018; Published online: 11 January 2019

Abstract
Glutathione S-transferases (GSTs) are multifunctional proteins involved in stress metabolism, which play major roles in biotic and abiotic stress responses. GSTs have found in all organisms which is a major phase II detoxification enzymes found in the cytosol. GSTs regulate peroxidase and isomerase activities, they protect cells against $H(_2)O(_2)$-induced cell death. Papaya rigsport virus (PRSV) is one of main biotic agent that cause damage in papaya. The disease symptoms are mosaic, chrolosis, ring spot and stunt, all the symptoms are the early stage of cell death. The main point of this research to investigate protein interaction of the PRSV interacted plant proteins and GSTs recombinant protein using the classical Affinity-purification-mass spectrometry (AP-MS) approache. The GST protein was heterologous expressed in *E.coli* system and the pull down assay was applied to expore the protein interacted complexs after that the protein complexs were indentified by LC-MS/MS. CTC1, Protein CCA1 isoform X1, Tetratricopeptide repeat (TPR)-like superfamily protein, PHD finger protein ALFIN-LIKE 9 and Fructose-bisphosphate aldolase-lysine N-methyltransferase proteins were identified. The interacted five proteins were predicted the protein network by STITCH program, the result show they associated with oxidative stress response mechanism. However, this is the basic intensive information that could develop to manufacture PRSV resistance variety in the future.

Keywords: *Carica papaya*, Glutathione S-transferase, AP-MS, Protein-protein interactions

Please cite this article as: A. Sawwa, Y. Yingchutrakul, S. Roytrakul, W. Siriwan, and S. Chowpongpang, "Identification of GST interacted proteins under PRSV infected papaya using affinity purification–mass spectrometry," *Applied Science and Engineering Progress*, vol. 13, no. 3, pp. 224–232, Jul.–Sep. 2020.

1 Introduction

Papaya (*Carica papaya* L.) is planted throughout tropical and sub-tropical regions. It is a popular and economically important fruit tree, but it is severely affected worldwide by Papaya ringspot disease [1]. This disease caused by *Papaya ringspot virus* (PRSV) is severely damage to papaya crops and seriously limits papaya production, because the infected plants are stunted and reduced fruit yield [2]–[4]. PRSV has been recognized as a destructive disease in many areas including Thailand resulting in the decline in fruit production. The first report of PRSV was appeared in the Northeast in the 1970s and now severely affects papaya all over Thailand [5].

Glutathione S-transferases (GSTs) are multifunctional proteins encoded by a large gene family, appear to be ubiquitous in plants and are involved in stress response during biotic and abiotic stress response [6], [7]. GSTs catalyze the conjugation of tripeptide glutathione (GSH) with endogenous electrophilic compounds (secondary metabolites, hydroperoxides) and xenobiotics, such as herbicides, leading to their cellular detoxification [8]. GSTs safeguard the cells against chemical-induced toxicity and provide tolerance by catalyzing S-conjugation between the thiol group of GSH and electrophilic moiety in the hydrophobic and toxic substrate. After conjugation, the conjugate is either sequestered into the vacuoles or are exported from the cells by putative membrane ATP-dependent pump systems [9].

The study of protein-protein interactions (PPIs) is a critical element of biological systems, and the analysis of interaction partners can provide valuable hints about unknown functions of a protein [10]. Identifying the interactions established by a protein of interest can be a critical step in understanding its function when an unknown protein of interest is demonstrated to physically interact with proteins of known function. Many a time, when studying protein complexes rather than individual proteins, the biological insight gain has been fundamental, particularly in cases in which proteins with no previous functional annotation [11]. While many techniques have been developed to characterize PPIs, one strategy, is affinity-purification followed by mass spectrometry (AP-MS) [12]. AP-MS is a result of improved methods to enrich samples and perform separation chromatography as advances in the resolution and sensitivity of mass spectrometers. The AP-MS studies produce rich of information data that detail protein-protein interaction to help characterize the function of proteins, and can reveal networks of biological processes [13]. The network represents an important aspect of systems biology. The understanding of the PPIs network and interactome will provide crucial insights into the regulation of plant developmental, physiological, and pathological processes [14], [15]. However, substantial recent advance in the graph-theoretical functional interpretation of complex network architectures might pave the way for novel approaches in plant research [16]. Therefore, this study used pull-down assay combined with AP-MS to understand the function of proteins in papaya after PRSV infection. The GST interactome network of papaya proteins were discussed for their potential biological roles.

2 Materials and Methods

2.1 *Plant materials and virus sources*

Papaya Carica, Khak-Dam Srisaket variety seeds were grown in a greenhouse until 2 months, after that the papaya pots were placed in a versatile environmental test chamber (MLR-350HR, SANYO) set to15,000 lx of the light intensity, 8 h of light and 16 h of dark, the temperature at 30°C during light period and 25°C during dark period, and humidity at 75% under either long day [17] for 3 days before PRSV inoculation. One gram of leaf tissues from PRSV infected were ground in a mortar containing 0.02 M phosphate buffer (pH 7.0). The mechanical inoculations were performed on leaves that dusted with silicon carbide abrasive (Carborundum). The PRSV infection was compared with a control (healthy papaya), it was dusted only with carborundum and phosphate buffer. The samples were collected at 20 days post-inoculation. A total of six plants were used for each treatment and the plant leaves were collected and frozen immediately in liquid nitrogen then stored at –80°C until extracting protein.

2.2 *Protein expression and purification*

The pGEX-4T-1 plasmid construct was transformed into *E.coli* (BL21DE3) cells to express GST recombinant protein. Expression analysis experiments were started in 50 mL baffled shaking flasks with 25 mL LB (Luria-

Bertani) Broth, Miller (DIFCO® Dehydrated Culture Media and Ingredients, UK) containing 50 µg/mL ampicillin. The single colony of BL21DE3 transformant was pre-cultured overnight with 120 rpm shaking at 30°C. The 0.1% of starters was cultured in a new baffled shaking flask with 25 mL LB plus 50 µg/mL ampicillin to a starting OD 600 nm at 0.4. The IPTG was then added to final concentration of 1 mm for induction, and the bacterial cells were harvested after growing for 0, 2, 4, 8, and 16 h at 120 rpm at 30°C. Crude proteins were separated by SDS-PAGE (NuPAGE Bis-Tris Gels, Thermo Fisher Scientific, USA) to observe the expected protein according to size at 27.9 kDa. After optimization,the 200 mL cultured cells induced with 1 mm IPTG for 8 h were harvested and the bacterial pellets were flash frozen in liquid nitrogen and kept at –80°C until further use for purification and pull-down assays. Bacterial pellets were suspended in 5 mL of lysis buffer (50 mm Tris, 150 mm NaCl, pH 8.0; 5 mm DDT; 0.1 mm PMSF) and disrupted by sonication using 130 W Ultrasonic Processor (Sonics vibra cell ,VCX 130 PB/VCX 130 FSJ, USA) for 5 min (10 s sonicating followed by 10 s pause) in an ice-water beaker. Cell lysates was centrifuged at 4°C, 12,000 g for 10 min, the soluble recombinant GST protein was purified using 20 mg of Glutathione Agarose (Pierce®, Thermo Fisher Scientific, USA). Briefly, the fraction was mixed with 10 resin-bed volumes of Equilibration/Wash buffer (50 mm Tris, 150 m NaCl, pH 8.0), shaked on ice for 30 min followed by packed in an empty column. The column pre-equilibrated with GST protein were washed with 5 volumes of Equilibration/ Wash buffer. GST tagged protein were eluted with 2 volumes of elution buffer (50 mm Tris, 150 mm NaCl, pH 8.0 containing 10 mm reduced glutathione). Protein concentration of each fraction was monitored by Coomassie Plus (Bradford) Assay Kit (Thermo Fisher Scientific, USA) using BSA as protein standard.

2.3 *Preparation of plant protein extracts*

One gram of frozen papaya leaves were ground in liquid nitrogen using mortar and pestle. The ground leaves were extracted in 5 mL of 20 mm Tris buffer pH 7.5, 150 mm NaCl, 2 mm $MgCl_2$, 0.5 mm DTT, 5% glycerol, 1% PVPP (w/v) [18] supplemented with protease inhibitor, 1X of protease inhibitor cocktail kit (Thermo Fisher Scientific, USA) and 1 mm PMSF. Plant extracts were centrifuged at 4°C, 10,000 g for 10 min. Any particulates were further eliminated from the supernatant by filtration through a 0.22 µm syringe filter. Protein concentration (1 mg/mL) was determined using Coomassie Plus (Bradford) Assay KitSamples were kept on ice and used for pull-down assays immediately.

2.4 *Protein pull-down assay*

The GST protein expressed in *E.coli* BL21(DE3) was used as a bait to trap protein that interacts. To starting pull-down assays, the GST immobilized glutathione agarose matrix was washed with Equilibration/Wash buffer, and mixed with pray proteins, 1 mg of whole papaya proteins (healthy and infected papayas) in 5 mL of 1x PBS buffer including 1 mm PMSF. The interacted proteins were incubated at 4°C with gentle rolling for 2 h, packed into empty column, and washed with Equilibration/Wash buffer. The proteins complexes were eluted with 2 volumes of elution buffer, and desalted with dialysis (SnakeSkin™ Dialysis Tubing, 10 K MWCO, 22 mm, Thermo Fisher Scientific, USA) in 1 L of 1xPBS buffer for 1 h at 4°C with magnetic bar stirred; repeat this step twice for overnight. The protein concentration of complex fraction was measured by Coomassie Plus (Bradford) Assay kit before LC-MS/MS analysis.

2.5 *Nano- liquid Chromatography Mass spectrophotometry (Nano LC-MS/MS)*

The eluted proteins complexes were determined by Lowry assay using BSA as a standard protein. In-solution digestion, each complex protein was hydrolyzed by trypsin at an enzyme to protein ratio of 1 : 50 at 37°C for 24 h. Then the peptides were dried by vacuum centrifuge. The extracted peptides were dissolved in 15 µL of 0.1% formic aid, centrifuged at 10,000 rpm for 10 min and separated using an Ultimate 3,000 LC System (Dionex Ltd., U.K.) on a nanocolumn PepSwift monolithic column (100 mm i.d.,650 mm), with a flow rate of 300 nl/min using multi-steps gradient of a linear concentration increase from 10 to 90% of 80% acetonitrile in 0.1% formic acid within 20 min. The nanoLC system was connected with an electrospray interface with ESI-Ion Trap MS (Bruker Daltonik GmbH, Bremen, Germany).

2.6 *Protein quantitation and identification*

Protein quantitation was performed with DeCyder MS Differential Analysis software (DeCyderMS, GE Healthcare). Acquired LC–MS raw data were converted, and the PepDetect module was used for automated peptide detection, charge state assignments, and quantitation based on the peptide ions signal intensities in MS mode. The analyzed MS/MS data from DeCyderMS were submitted to a database search using the Mascot software (Matrix Science, London, UK). The data were searched against the NCBI database for protein identification. Database interrogation was taxonomy (Plant), enzyme (trypsin), variable modifications (carbamidomethyl, oxidation of methionine residues), mass values (monoisotonic), protein mass (unrestricted), peptide mass tolerance (1.2 Da), fragment mass tolerance (60.6 Da), peptide charge state (1+, 2+, and 3+) and mass missed cleavages. Proteins considered as identified proteins had at least one peptide with an individual mascot score corresponding to $p < 0.05$. The expression level was presented in log2 value. The biological and molecular functions of the matched protein were assigned to protein identification according to the Uniprot (http://uniprot.org). Protein-protein interaction was analyzed according to STITCH 5.0 database (http://stitch.embl.de/) [19].

3 Results and Discussion

3.1 *Expression and purification protein*

The pGEX-4T-1 vector was transformed into *E.coli* (BL21DE3) and induced with 1 mm IPTG at 30°C for 0, 2, 4, 8, and 16 h. The soluble crude proteins were separated by SDS-PAGE, the target 27.9 kDa GST protein band was detected after induction for 2 h [Figure 1(a)]. In order to get high amount of GST with less cell debris, eight hours post-IPTG induced bacteria was harvested and purified using glutatathione agarose matrix. Purity of the 27.9 kDa of GST bait was estimated by SDS-PAGE as shown in Figure 1(b).

3.2 *Pull down assay and LC-MS/MS analysis*

The pull down proteins assays were performed by studying the binding of papaya protein with immobilized GST recombinant protein. The total protein of heathy and infected papaya were extracted and gently mixed with GST recombinant protein. Nonspecifically bound proteins were washed out, while specific bound protein were captured. Protein partners were eluted from resin and tryptic digested before analysis by LC-MS/MS. Seven proteins were founded to interact with GST recombinant protein. They are CTC1 (gi|1032284868), Protein CCA1 isoform X1 (gi|1072981418), Tetratricopeptide repeat (TPR)-like superfamily protein (gi|42566234), PHD finger protein ALFIN-LIKE 9 (gi|685308104) and Fructose-bisphosphate aldolase-lysine N-methyltransferase (gi|729450654), the Hypothetical protein (gi|557103554) and Uncharacterized protein (gi|729356814) (Table 1). No protein from healthy papaya was bound to GST protein. The plant GSTs involved in biotic and abiotic stress response, such as pathogen attack and fungal elicitors [20], herbicides [21], high temperature [22], dehydration [23], UV light [24], cold [25] heat shock, and high salt, and hormone treatment [26], [27]. GST in papaya might take action in response to PRSV infection. The biological functions of the identified GST binding proteins were DNA binding, translation, transcription and photosynthesis.

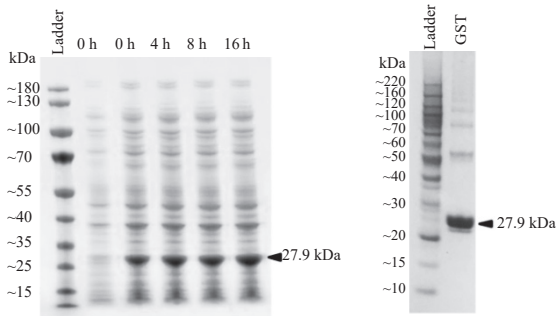

Figure 1: The protein expression, (a) GST inclusion from BL21(DE3) induced by 1 mm IPTG for 0, 2, 4, 8, and 16 h at 30°C, (b) The purified GST Protein using glutathione agarose bead.

The tetratricopeptide repeat protein was involved in translation function [28]. On the other hand, the fructose-bisphosphate aldolase-lysine N-methyltransferase was involved in photosynthesis [29]. There are three proteins involved in DNA binding and transcription functions. Firstly, CTC1 is a component of the CST (CTC1-STN1-TEN1) complex, a complex that binds to single-stranded DNA and is required to protect telomeres from DNA degradation. The absence of CST

Table 1: The identified papaya proteins that interacted with recombinant GST protein between healthy and PRSV infected papaya

Accession No.	Protein Name	Function	ID Score	Peptide	Healthy Papaya: GST	Infected Papaya: GST
gi|1032284868	CTC1	DNA binding [30]	30.56	GALCLPK	ND*	19.75619**
gi|557103554	Hypothetical protein (Unknown)	Unknown	18.86	GLDQSDIVVSSR	ND	16.31491
gi|729356814	Uncharacterized protein	Unknown	14.73	QTKGSSKTSAK	ND	15.38716
gi|729450654	Fructose-bisphosphate aldolase-lysine N-methyltransferase	Photosynthesis [29]	14.09	ATAASAAMALLR	ND	15.27027
gi|42566234	Tetratricopeptide repeat (TPR)-like superfamily protein	Translation [28]	12.79	EGKLEGAVK	ND	16.4012
gi|685308104	PHD finger protein ALFIN-LIKE 9	Transcription [36] and Stress response [37]	9.22	PPVKASGR	ND	16.88851
gi|1072981418	Protein CCA1 isoform X1	DNA binding, Transcription [35], and Stress response [34]	8.21	RCSMEAKESSR	ND	15.47841

*ND: Not detect
**The log of the peptide intensity

in Arabidopsis triggers a multifaceted ATR (Ataxia Telangiectasia mutated and Rad3-related) response to facilitate maintenance of critically shortened telomeres and eliminate cells with severe telomere dysfunction [30]. CTC1 works in concert with TEN1 as a highly dynamic protein to preserve telomere integrity in response to environmental could be lead to genome instabilities [31], [32], and the mutations in CTC1 lead to the telomere syndromes [33]. Secondly, CCA1 (CIRCADIAN CLOCK-ASSOCIATED 1) is a timekeeper mechanism that is able to regulate timekeeper mechanism that is able to regulate biological activities with a period of 24 h. Proper matching of the internal circadian time with the environment not only confers fitness advantages but also allows the clock to temporally gate the responses to environmental stresses [34]. The CCA1 mutants *in Arabidopsis* revealed that CCA1 directly activates GLK2 and suppresses ORE1 expression to counteract leaf senescence. As plants age, the expression and periodic amplitude of CCA1 declines and thus weakens the inhibition of senescence [35]. Thirdly, PHD (Plant Homeodomain)-finger protein is a common structural motif found in all eukaryotic genomes, and known as key players in regulating transcription and chromatin structure. It is a Zn^{2+}-binding domain and its closest structural relative is the RING domain [36], [37].

3.3 *STITCH analysis of GST protein interaction network*

The knowledge about interactions between proteins and small molecules is essential for the understanding of molecular and cellular functions. STITCH (Search Tool for Interactions of Chemicals) is a resource to explore known and predicted interactions of chemicals and proteins [38]. This enables the researcher to get a quick overview of the potential effects of the chemical on its interaction partners [39]. The association between GST protein and five interacting proteins (CTC10, CCA1, TPR, PHD finger protein and Fructose-bisphosphate aldolase-lysine N-methyltransferase) were predicted by STITCH version 5.0 using the parameters followed as the organism of *Arabidopsis thaliana*, medium confidence score (0.4) and active prediction methods (no more than 10 interactions).The results showed that the identified protein are associated with proteins involved in DNA binding, translation, transcription and photosynthesis (Figure 2).

The GST was associated with PHD finger protein (AT3G61723) and conserved telomere maintenance component 1 (CTC1) which are known to involve in transcription. The study by Wang *et al.* identified 67 non-redundant PHD members in the maize genome and found that *ZmPHD14* and *ZmPHD19* are likely

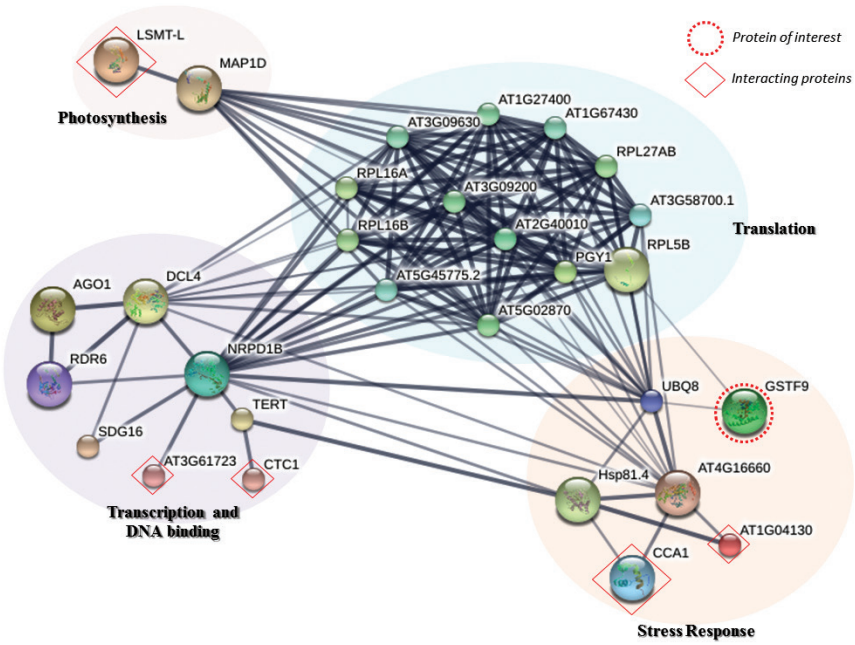

Figure 2: The network of identified five proteins predicted by STITCH database based on the following analysis parameters; species (*Arabidopsis thaliana*); medium confidence score (0.4) and active prediction methods (no more than 10 interaction). Abbreviation: Glutathione S-transferases (GSTF9), Conserved Telomere maintenance Component 1 (CTC1), Circadian Clock-Associated 1 (CCA1), Tetratricopeptide repeat (AT1G04130), PHD finger protein (AT3G61723), Fructose-bisphosphate aldolase-lysine (LSMT-L), RNA-dependent RNA polymerase 6 (RDR6), Dicer-like 4 (DCL4), Argonaut 1 (AGO1) Ubiquitin (UBQ8), Hypoxia up-regulated 1 (AT4G16660), Nuclear RNA polymerase D1B (NRPD1B), Heat shock protein (HSP81.4), Telomerase reverse transcriptase (TERT), 60S acidic ribosomal protein P0-1 (AT2G40010), 60S ribosomal protein L17-2 (AT1G67430), 60S ribosomal protein L4-1 (AT3G09630), 60S ribosomal protein L4-2 (AT5G02870), Ribosomal protein L5 (AT3G25520), 60S ribosomal protein L10a-1 (AT1G08360), 60S acidic ribosomal protein P1-3 (AT5G47700), 60S ribosomal protein L10a-3 (AT5G22440), SET domain protein (SDG16), Methionine aminopeptidase (MAP1D), Ribosomal protein L5 B (RPL5B) and PIGGYBACK1 (PGY1).

involved in the abiotic stress response [37]. The CTC1 was linked to Telomerase reverse transcriptase (TERT) and Heat shock protein (HSP81.4) which relevant in stresses response. The Fructose-bisphosphate aldolase-lysine N-methyltransferase (LSMT-L) interacted with methionine aminopeptidase (MAP1D) has the function to remove the N-terminal methionine from nascent proteins. In addition, the tetratricopeptide repeat (AT1G04130) and CCA1 showed interaction with Hypoxia up-regulated 1 (AT4G16660). The hypoxia is a decline in oxygen availability. In Arabidopsis, the hypoxia up-regulated protein was found in root [40]. Molecular-oxygen deficiency leads to altered cellular metabolism and can dramatically reduce crop productivity [41].

GST and its interacting proteins were associated with the stress response which connects with Ubiquitin (UBQ8), Hypoxia up-regulated 1 (AT4G16660) and Heat shock protein (HSP81.4). Fang *et al*. conducted transcriptome sequencing of PRSV resistant transgenic papaya SunUp and its PRSV susceptible progenitor Sunset to compare the transcriptional changes in young healthy leaves prior to infection with PRSV. They found that the expression pattern of the *GST* gene was upregulated after PRSV infection, which revealed that the *GST* can stress-induced by PRSV [41]. In addition, this network showed association of the RDR6, AGO1 and DCL4 which involved in RNA

silencing. However, further studies are necessary to elucidate the exact functions of the large GST family in infected plants. Five protein obtained from AP-MS was found to interacted with protein involved in abiotic and biotic stress response as not only reported by many researchers but also predicted by STITCH. It indicated that PRSV infected papaya might fight against PRSV by GST mediated defense mechanism.

4 Conclusions

The protein complex of purified GST protein and interacting proteins from whole leaves of healthy and PRSV infected were examined using AP-MS method, and STICTH. Seven interacting proteins were found only in PRSV infected papaya. Five proteins with known functions, are CTC1, CCA1, TPR, PHD finger protein and Fructose-bisphosphate aldolase-lysine N-methyltransferase. Interestingly, all five interacting proteins were associated to the proteins which related in stress responses. Therefore, the GST protein and its interacting proteins provided more understanding of the stress responsive protein under PRSV infection.

Acknowledgments

This research is 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)

References

[1] R. Lines, D. Persley, J. Dale, R. Drew, and M. Bateson, "Genetically engineered immunity to *Papaya ringspot virus* in Australian papaya cultivars." *Molecular Breeding*, vol. 10, pp. 119–129, Sep. 2002.

[2] S. U. Chalak, S. N. Hasbnis, and V. S. Supe, "Papaya ringspot disease management: A review," *Journal of Pharmacognosy and Phytochemistry*, vol. 6, no. 5, pp. 1911–1914, Aug. 2017.

[3] S. D. Yeh and D. Gonsalves, "Practices and perspective of control of *Papaya ringspot virus* by cross protection," in *Advances in Disease Vector*, New York: Springer, 1994, pp. 237–257.

[4] D. Gonsalves, J. Y. Suzuki, S. Tripathi, and S. A. Ferreira, "Papaya ringspot virus," in *Encyclopedia of Virology*. 3rd ed., Amsterdam, Netherlands: Elsevier Ltd., 2008, pp. 1–8.

[5] S. Sakuanrungsirikul, N. Sarindu, V. Prasartsee, S. Chaikiatiyos, R. Siriyan, M. Sriwatanakul, P. Lekananon, C. Kitprasert, P. Boonsong, P. Kosiyachinda, G. Fermin, and D. Gonsalves, "Update on the development of virus-resistant papaya: Virus-resistant transgenic papaya for people in rural communities of Thailand," *Food and Nutrition Bulletin*, vol. 26, no. 4, pp. 422–426, Dec. 2005.

[6] E. Nutricati, A. Miceli, F. Blando, and L. De Bellis, "Characterization of two *Arabidopsis thaliana* glutathione S-transferases," *Plant Cell Reports*, vol. 25, pp. 997–1005, Sep. 2006.

[7] S. Mohsenzadeh, M. Esmaeili, F. Moosavi, M. Shahrtash, B. Saffari, and H. Mohabatkar, "Plant glutathione S-transferase classification, structure and evolution," *African Journal of Biotechnology*, vol. 10, no. 42, pp. 8160–8165, Aug. 2011.

[8] E. Chronopoulou, N. Georgakis, I. Nianiou-Obeidat, P. Madesis, F. Perperopoulou, F. Pouliou, E. Vasilopoulou, E. Ioannou, F. S. Ataya, and N. E. Labrou, "Plant glutathione transferases in abiotic stress response and herbicide resistance," in *Glutathione in Plant Growth, Development, and Stress Tolerance*. New York: Springer, 2017, pp. 215–233.

[9] S. Kumar and P. K. Trivedi, "Glutathione S-transferases: Role in combating abiotic stresses including arsenic detoxification in plants," *Frontiers in Plant Science*, vol. 9, p. 751, Jun. 2018.

[10] P. Braun, A. Sebastien, V. L. Jelle, D. J. Geert, and L. Claire, "Plant protein interactomes," *Annual Review of Plant Biology*, vol. 64, no. 1, pp. 161–187, Jan. 2013.

[11] A. Bauer and B. Kuter, "Affinity purification-mass spectrometry. Powerful tools for the characterization of protein complexes," *European Journal of Biochemistry*, vol. 270, no. 4, pp. 570–578, Feb. 2003.

[12] W. H. Dunham, M. Mullin, and A. C. Gingras, "Affinity-purification coupled to mass spectrometry: Basic principles and strategies," *Proteomics*, vol. 12, no. 10, pp. 1576–1590, May. 2012.

[13] J. H. Morris, G. M. Knudsen, E. Verschueren, J. R.

Johnson, P. Cimermancic, A. L. Greninger, and A. R. Pico, "Affinity purification-mass spectrometry and network analysis to understand protein-protein interactions," *Nature Protocol*, vol. 9, no. 11, pp. 2539–2554, Oct. 2014.

[14] Y. Zhang, P. Gao, and J. S. Yuan, "Plant protein-protein interaction network and interactome," *Current Genomics*, vol. 11, no. 1, pp. 40–46, Mar. 2010.

[15] Y. Fukao, "Protein-protein interactions in plants," *Plant and Cell Physiology*, vol. 53, pp. 617–625, Apr. 2012.

[16] F. U. Joachim, "Protein interaction networks in plants," *Planta*, vol. 224, pp. 771–781, Mar. 2006.

[17] J. Chumpookam, H. L. Lin, and C. C. Shiesh, "Effect of smoke-water on seed germination and seedling growth of papaya (*Carica papaya* cv. Tainung no. 2)," *HortScience*, vol. 47, pp. 741–744, Jun. 2012.

[18] H. G. Pennington, D. M. Gheorghe, A. Damerum, C. Pliego, P. D. Spanu, R. Cramer, and L. V. Bindschedler, "Interactions between the powdery mildew effector BEC1054 and barley proteins identify candidate host targets," *Journal of Proteome Research*, vol. 4, pp. 826–839, Feb. 2016.

[19] D. Szklarczyk, A. Santos, C. Mering, L. J. Jensen, P. Bork, and M. Kuhn, "STITCH 5: Augmenting protein–chemical interaction networks with tissue and affinity data," *Nucleic Acids Research*, vol. 44, pp. 380–384, Nov. 2016.

[20] S. Harper and D. W. Speicher, "Purification of proteins fused to glutathione S-tranferase," *Methods in Molecular Biology*, vol. 681, pp. 259–280, Feb. 2011.

[21] R. Edwards, D. P. Dixon, and V. Walbot, "Plant glutathione S-transferases: Enzymes with multiple functions in sickness and in health," *Trends Plant Science*, vol. 5, pp. 193–198, May 2000.

[22] E. Chronopoulou, N. Georgakis, I. Nianiou, P. Madesis, F. Perperopoulou, F. Pouliou, E. Vasilopoulou, E. Loannou, F. S. Ataya, and N. Labrou, "Plant glutathione transferases in abiotic stress response and herbicide resistance," in *Glutathione in Plant Growth, Development, and Stress Tolerance*. New York: Springer, 2017, pp. 215–233.

[23] H. Gong, Y. Jiao, W. Hu, and E. Pua, "Expression of glutation-S-transferase and its role in plant growth and development *in vivo* and shoot morphogenesis in vitro," *Plant Molecular Biology*, vol. 57, pp. 53–66, Jan. 2005.

[24] T. Kiyosue, K. Yamaguchi-Shinozaki, and K. Shinozaki, "Characterization of two cDNAs (ERD11 and ERD13) for dehydration-inducible genes that encode putative glutathione S-transferase in *Arabidopsis thaliana* L," *FEBS Letters*, vol. 335, pp. 189–192, Dec. 1993.

[25] L. Loyall, K. Uchida, S. Braun, M. Furuya, and H. Frohnmeyer, "Glutathione and a UV light-induced glutathione S-transferase are involved in signaling to chalcone synthase in cell cultures," *Plant Cell*, vol. 12, pp. 1939–1950, Oct. 2000.

[26] J. V. Anderson and D. G. Davis, "Abiotic stress alters transcript profiles and activity of glutathione S-transferase, glutathione peroxidase, and glutathione reductase in Euphorbia esula," *Physiologia Plantarum*, vol. 120, pp. 421–433, Feb. 2004.

[27] J. Zhou and P. B. Goldsbrough, "An *Arabidopsis* gene with homology to glutathione S-transferase is regulated by ethylene," *Plant Molecular Biology*, vol. 22, pp. 517–523, Mar. 1993.

[28] N. Zeytuni and R. Zarivach, "Structural and functional discussion of the Tetra-Trico-Peptide repeat, a protein interaction module," *Structure*, vol. 20, no. 3, pp. 397–405, Mar. 2012.

[29] M. Mininno, S. Brugière, V. Pautre, A. Gilgen, S. Ma, M. Ferro, M. Tardif, C. Alban, and S. Ravanel, "Characterization of chloroplastic fructose 1,6-bisphosphate aldolases as lysine-methylated proteins in plants," *The Journal of Biological Chemistry*, vol. 287, no. 25, pp. 21034–21044, Apr. 2012.

[30] K. A. Boltz, K. Leehy, X. Song, A. D. Nelson, and D. E. Shippen, "ATR cooperates with CTC1 and STN1 to maintain telomeres and genome integrity in *Arabidopsis*," *Molecular Biology of the Cell*, vol. 23, pp. 1558–1568, Feb. 2012.

[31] J. R. Lee, X. Xie, K. Yang, J. Zhang, S. Y. Lee, and D. E. Shippen, "Dynamic interactions of *Arabidopsis* TEN1: Stabilizing telomeres in response to heat stress," *The Plant Cell*, vol. 28, no. 9, pp. 2212–2224, Sep. 2016.

[32] Y. Wang and W. Chai, "Pathogenic CTC1 mutations cause global genome instabilities under replication stress," *Nucleic Acids Research*, vol. 46, no. 8, pp. 3981–3992, May 2018.

[33] L. Chen, J. Majerska, and J. Lingner, "Molecular basis of telomere syndrome caused by CTC1 mutations," *Genes and Development*, vol. 27, pp. 2099–2108, Sep. 2013.

[34] P. J. Seo and P. Mas, "Stressing the role of the plant circadian clock," *Trends in Plant Science*, vol. 20, pp. 230–237, Apr. 2015.

[35] Y. Song, Y. Jiang, B. Kuai, and L. Li, "Circadian clock-associated 1 inhibits leaf senescence in *Arabidopsis*," *Frontiers in Plant Science*, vol. 9, pp. 280, Mar. 2018.

[36] M. Bienz, "The PHD finger, a nuclear protein-interaction domain," *Trends in Biochemical Sciences*, vol. 31, pp. 35–40, Jan. 2006.

[37] Q. Wang, J. Liu, Y. Wang, Y. Zhao, H. Jiang, and B. Cheng, "Systematic analysis of the maize PHD-finger gene family reveals a subfamily involved in abiotic stress response," *International Journal of Molecular Sciences*, vol. 16, no. 10, pp. 23517–23544, Oct. 2015.

[38] M. Kuhn, C. Mering, M. Campillos, L. J. Jensen, and P. Bork, "STITCH: Interaction networks of chemicals and proteins," *Nucleic Acids Research*, vol. 36, pp. D684–D688, Dec. 2008.

[39] M. W. Hameed, "Hypoxia up-regulates mitochondrial genome-encoded transcripts in *Arabidopsis* roots," *Genes and Genetic Systems*, vol. 90, pp. 325–334, Apr. 2015.

[40] T. Fukao and J. Bailey-Serres, "Plant responses to hypoxia; is survival a balancing act," *Trends in Plant Science*, vol. 9, no. 9, pp. 449–456, Sep. 2004.

[41] J. Fang, A. Lin, W. Qiu, H. Cai, M. Umar, R. Chen, and R. Ming, "Transcriptome profiling revealed stress-induced and disease resistance genes up-regulated in PRSV resistant transgenic papaya," *Frontiers in Plant Science*, vol. 7, p. 855, Jun. 2016.