Published in final edited form as:
J Proteomics. 2012 June 18; 75(11): 3191–3198. doi:10.1016/j.jprot.2012.03.021.

Label-free quantitative proteomics reveals differentially regulated proteins in the latex of sticky diseased *Carica papaya* L. plants

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Abstract

Papaya meleira virus (PMeV) is so far the only described laticifer-infecting virus, the causal agent of papaya (*Carica papaya* L.) sticky disease. The effects of PMeV on the laticifers' regulatory network were addressed here through the proteomic analysis of papaya latex. Using both 1-DE- and 1D-LC-ESI-MS/MS, 160 unique papaya latex proteins were identified, representing 122 new proteins in the latex of this plant. Quantitative analysis by normalized spectral counting revealed 10 down-regulated proteins in the latex of diseased plants, 9 cysteine proteases (chymopapain) and 1 latex serine proteinase inhibitor. A repression of papaya latex proteolytic activity during PMeV infection was hypothesized. This was further confirmed by enzymatic assays that showed a reduction of cysteine-protease-associated proteolytic activity in the diseased papaya latex. These findings are discussed in the context of plant responses against pathogens and may greatly contribute to understand the roles of laticifers in plant stress responses.

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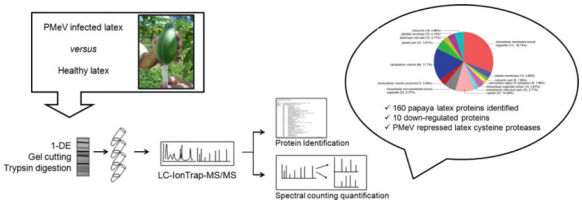
Supplementary data

Additional information about data normalization and GO grouping graphics are available in a pdf file. A complete list of identified peptides, the corresponding spectral count information as well as the annotation details are available in Microsoft Excel sheets.



Keywords

Carica papaya; Label-free quantitative proteomics; Latex; Mass spectrometry; Plant proteomics

1. Introduction

Laticifers are cells that produce and store latex, a complex fluid which contains a variety of secondary metabolites and proteins [1]. Laticifers have been reported in forty plant families, including *Carica papaya* L. The latex of this specie presents high titles of cysteine endopeptidases, *i.e.*, papain, chymopapain, glycyl endopeptidase, and caricain [2], which are part of the plant defense repertoire against herbivorous [3], and supposedly, phytopathogens [1, 4].

Despite the presence of proteases in papaya latex, a dsRNA virus named *Papaya meleira virus* (PMeV) was described in both latex and laticifers of the plant [5, 6]. PMeV infection results in "papaya sticky disease" or "meleira", characterized by an exudation of fluid and translucent latex from the fruits and leaves. The latex oxidizes after atmospheric exposure, resulting in small necrotic lesions on the edges of young leaves and a sticky latex on the plant organs that makes the fruits unacceptable for consumption [6, 7]. The disease also interferes with the natural resistance of papaya fruits to fruit flies (Diptera: Tephretidae), which are significant pests of fruit production worldwide with a quarantine importance [7].

PMeV is the only virus that has been described in laticifers [5, 6, 8]. Nevertheless, the proteomic analysis of lettuce (*Lactuca sativa*) latex revealed that several virus-related proteins were among the most abundant identified proteins [9], suggesting that other, yet unidentified viruses might be infecting the laticifers of other species.

Recently, we have demonstrated that stress responsive proteins, mostly calreticulin and proteasome-related proteins, are up-regulated in sticky diseased papaya leaf tissues [10], supporting the assumption that PMeV proteins or even host proteins essential for the viral infection could be targeted to degradation. This mechanism has been shown to constitute part of the resistance response of plants against viruses, as recently reviewed [11]. However, the specific responses of papaya laticifers to PMeV infection are still poorly understood.

Previous studies demonstrated the accumulation of calcium oxalate crystals in the infected papaya latex positively correlated with the increased production of hydrogen peroxide (H_2O_2) in the laticifers of the plant [8]. This may be an indicative of hypersensitive response (HR) of papaya laticifers against PMeV infection. HR is usually triggered after infection of plants by microbial pathogens and is often accompanied by a form of programmed cell death (PCD) in and around the initial infection site [12]. Several proteases are known as important players of HR/PCD in plants [13–15]. Nevertheless, the involvement of papaya latex proteases in these processes remains elusive.

In healthy papaya plants, the cysteine proteases of latex are activated a few seconds after tapping of laticifers and the consequent latex exudation [16]. Curiously, the exudation process is spontaneous in sticky diseased papaya [6, 7]. Increased levels of potassium,

phosphorous, and water were observed in the PMeV-infected latex, suggesting that the spontaneous exudation process may be a result of an osmotic disturbance of the laticifers. In addition, increased production of H_2O_2 in the laticifers and reduced calcium levels in the latex were reported in PMeV-infected papaya [8]. Since H_2O_2 and calcium are modulators of stress responses in plants [17], we addressed the effect of the virus on the stress regulatory networks of papaya laticifers.

Here, we carried out the proteomic analysis of *C. papaya* latex and identified a total of 160 non-redundant proteins, of which 122 had not been previously reported. Based on quantitative label-free data and biological assays, we demonstrated a reduction in proteolytic activity of papaya latex during sticky disease, specifically associated with cysteine proteases present in the latex.

2. Materials and methods

2.1 Sample preparation

Latex from *C. papaya* cultivar Sunrise (a commercial variety widely cultivated in Brazil) plants (n = 6) showing typical sticky disease symptoms [6, 7] and positive for molecular PMeV diagnosis [18, 19] was collected after single tapping of green fruits. Latex from healthy fruits was used as control. The samples were immediately frozen in dry-ice, lyophilized and pooled. For proteases assays, the lyophilized samples were resuspended using specific buffers as described below. Alternatively, the pellets were resuspended at 16.6 μg/μL in 100 mM Tris-HCl pH 7.6 containing 100 μM E-64 (N-trans-epoxysuccinyl-L-leucine 4-guanidinobutylamide, Sigma # E3132) at 4 °C and immediately centrifuged (12,000 × *g*, 15 min at 4 °C). The total protein concentration in the supernatant was assayed using Bradford reagent [20] (Table S1) and 40 μg were used for proteomic experiments.

2.2 Protein separation and digestion

The proteins were separated by 12.5% SDS-PAGE [21] and stained with Coomassie Brilliant Blue (CBB) [22]. The gel was sliced in 1 mm regular sections (5 lanes from control and 4 from infected latex) and washed two times using 25 mM ammonium carbonate/acetonitrile (1:1 v/v) at 25 °C for 15 hr. After removal of the wash solution, they were covered with acetonitrile for 20 min at 25 °C. The acetonitrile was removed, and the samples were allowed to dry. The dehydrated gel particles were submitted to protein reduction (10 mM dithiothreitol (DTT) at 56 °C for 30 min) and alkylation (50 mM iodoacetamide (IAA) at 25 °C for 30 min) and dried again. Protein digestion was conducted using 2 ng/μL trypsin (Sigma #T6567) in digest buffer (25 mM ammonium carbonate pH 8.0) for 20 hr at 37 °C. The resulting tryptic peptides were extracted two times with 50 μL of 0.1% (v/v) trifluoroacetic acid (TFA) in 50% (v/v) acetonitrile with 20 min sonication. The products from the two extractions were combined, vacuum dried and then dissolved in 0.1% (v/v) formic acid (FA) in water [23].

2.3 1D-LC-ESI-MS/MS analysis

After the trypsin digestion of proteins, resulting peptides were desalted using an in-house reverse-phase microcolumn and dried in a vacuum centrifuge [10]. Peptides were solubilized in 20 μL of mobile phase A (2% (v/v) acetonitrile and 0.1% (v/v) formic acid), and 5 μL was injected into a trap column (1 cm × 75 μm, 5 μm 100 Å, C18, Luna, Phenomenex). LC separation was performed using a reverse phase capillary column (20 cm × 75 μm, 5 μm 100 Å, C18, Luna, Phenomenex), connected to a nano-HPLC system (nano-LC 1D plus, Eksigent). The elution was carried out in a linear gradient from 0 to 40% solvent B (80% (v/v) acetonitrile and 0.1% (v/v) formic acid) over 100 min. The eluted peptides were directly introduced into an ESI-linear ion trap-mass spectrometer (LTQ XL, Thermo-Fisher

Scientific) equipped at the front end with a nanospray source (TriVersa NanoMate system, Advion). MS spectra were collected in centroid mode at the 400–1,700 *m/z* range, and the ten most abundant ions were submitted twice to collision-induced dissociation (35% normalized collision energy) before being dynamically excluded for 120 sec.

2.4 Protein identification and differential abundance analysis

MS/MS spectra derived from peptides having a mass range of 600–4,000 Da, and at least 15 fragments were submitted for database search using TurboSequest [24] (available in Bioworks version 3.3.1). The protein database used sequences from *C. papaya* (887 original protein sequences and 76,835 translated EST sequences, Jan 13th 2010). The ESTs were translated using OrfPredictor (http://proteomics.ysu.edu/tools/OrfPredictor.htmL). The databank also included *A. thaliana* (153,355, Jan 23th 2010), plant virus (37,014, Feb 23th 2010), keratin (1,091) and trypsin (01) sequences. All sequences (total of 269,183) were used in the forward and reverse orientations to calculate the false discovery rate (FDR). The database search parameters included: (i) trypsin cleavage in both peptide termini, allowing for one missed cleavage site; (ii) carbamidomethylation of cysteine residues as a fixed modification; (iii) oxidation of methionine residues as a variable modification; and (iv) 2.0 Da and 1.0 Da mass tolerance for peptides and fragments, respectively. The following filters were applied in Bioworks: DCn ≥ 0.85; protein probability ≤ 1×10^{-3}; and Xcorr ≥ 1.5, 2.0, and 2.5, for single-, double- and triple-charge peptides, respectively. After filtering, the files were exported into XML formats and the peptide sequences were assembled into proteins and redundant proteins into proteins groups (Table S2) using an in-house written Perl script [25]. Protein hits were re-filtered with sum of peptide Xcorr ≥3.5. The FDR was estimated to be < 1%, as described previously [26]. For the quantitative analysis, only the hit with the highest coverage of each group was used. With this approach we aimed to quantify groups of proteins with redundant functions, rather than individual sequences. Correlation and box plots between samples were down with DAnTE [27] and only the 3 most consistent replicates of each sample were used for quantitative analysis. Differentially abundant proteins were identified with QSPEC analysis of the spectral count data, which reported two different kinds of significance statistics: the odds of differential expression and the Z-statistic based on fold change parameter [28]. Multiple testing correction is performed by the false discovery rate estimation (FDR) procedure implemented in the new version of QSPEC (called QPROT). Proteins with significant differential expression between healthy and diseased latex were selected with the criterion FDR ≤ 0.1 (Table S3). All valid proteins were submitted to a gene ontology (GO) analysis using Blast2GO (www.blast2go.org/) [29]. The analysis was performed by searching (Blast-p) the identified protein sequences against the Swiss-Prot database. Only GOs with e-value ≤ $1.0E^{-3}$ for database search were considered.

2.5 Protease assay

Protease activity assay was performed as previously described [30], with minor modifications. Crude lyophilized latex (15 μg) was resuspended in 50 mM phosphate buffer pH 6.5 (0.5 mL final volume) and incubated at 37 °C for 5 min prior the addition of the same volume of 1% (w/v) casein solution. Both latex and casein were prepared using the same buffer. The reaction mixture was incubated for 30 at 37 °C and stopped by adding 0.5 mL of 10% (w/v) trichloroacetic acid (TCA). The samples were incubated 10 min at room temperature and centrifuged at 11,000 × *g* for 10 min. The supernatant was collected and mixed with an equal volume of 0.5 M NaOH solution, incubated for 15 min at RT and the absorbance measured at 440 nm in a Thermomax Microplate Reader (Molecular Devices, CA, USA). The mixture without latex was used as negative control. One unit of enzyme activity was defined as the amount of enzyme that produced an increase of 0.001 unit of absorbance at $A_{440\ nm}$ per 30 min in the above condition. The activation and inhibition of cysteine proteases was tested by the previous addition of 100 μM DTT or 5 μM E-64 to the

reaction mixture, respectively. The reaction was also performed using either 50 mM acetate pH 5.0 or 50 mM Tris-HCl pH 8.0.

3. Results

3.1 Identification of papaya latex proteins

The papaya latex presents high proteolytic activities and polymerizes right after tissue tapping what make its proteomic analysis very challenging. To overcome the proteolytic effects, the latex samples were immediately frozen in dry ice and lyophilized. Several conditions were tried (refs) in order to solubilize the latex proteins. The non-reproductive SDS-PAGE protein profiles observed (data not shown) suggested the proteins were being degraded. Alternatively, the proteins were digested using the filter-aided sample preparation [31], and analyzed by 2D-LC-ESI-MS/MS. However, just a few peptides could be identified (data not shown). After all those unsuccessful trials, we decided for solubilizing the latex proteins in the presence of 100 μM E-64, ten times more than usually recommended, and immediately separate the proteins by SDS-PAGE. Then, a complex protein profile could be observed (Fig. 1). This included a prominent protein band around 21 kDa in both, healthy and diseased samples, which represents the mixture of papaya cysteine endopeptidases (papain, caricain, papaya proteinase IV, and chymopapain), as previously described [2].

The MS/MS spectra (available at ProteomeCommons.org Tranche, hash: xEd +xkc5MB0w8eFvMtHZu/QQGGKZVLkAf4+nzPtdLq2Hn/fOmLZiVnQsoXft53gv1gyG3k N+Hoz5ekM4lSTLKqwDeOQAAAAAAAA1PA==) obtained from the gel sections were used to search against a protein database which included *C. papaya* EST and protein sequences available at NCBI. Searching MS/MS data against an EST database is a significant challenge since it includes many redundant, incomplete or missing sequences. To deal with the redundant sequences, the proteins were assembled then into proteins groups. The high abundance of cysteine proteases in papaya latex was another issue faced in this study. High abundant proteins considerably decrease the identification chances of low abundant proteins. Despite all difficulties,160 non-redundant papaya latex proteins were identified (FDR < 1%, Fig. 1 and Table S2 and S7). Among the identified proteins, 38 were previously reported in *C. papaya* latex (Table S4) whereas 122 represent newly identified proteins in the latex of this plant (Table 1). We also confirmed the presence of several isoforms of three putative proteins, *i.e.*, cystatin proteinase inhibitor [32], beta-1,3-glucanase [33], and lipase [34], that were previously supposed as occurring in papaya latex based on their homologous cDNA sequences (Table S4). The data also supported the annotation of 128 previously non-annotated papaya EST sequences (Table S3).

Blast2GO analysis provides a qualitative distribution of the annotated protein sequences in cellular components, biological processes, and molecular functions [29]. Blast2GO analysis predicted cytoplasmic vesicle (17.7%) and extracellular vesicle-/exosome-associated (3.6%) proteins in the papaya latex, although the majority of the proteins were most probably located in intracellular membrane-bound organelles (30.7%) and/or cytosol (10.2%, Fig. 2 and Table S5). This may suggest that these proteins could have been carried from the laticifers during the harvest of latex samples [4]. Proteolysis was one of the primary biological processes in the latex (9.4% of total proteins, Fig. S4 and Table S5), probably due to the fact that 18.7% of the proteins were associated with peptidase activity on L-amino acid peptides (Fig. S5 and Table S5). In terms of protein abundance, the proteases are known to represent ~32% of the latex dry mass [2]. Interestingly, several proteins (7.8 %) were predicted to be involved in developmental programmed cell death (Fig. S4). All proteins grouped in this biological process were cysteine proteases (Table S6).

3.2 Differential protein regulation revealed by label-free quantification

Diseased and healthy control latex proteins datasets were compared by quantitative analysis of normalized spectral count (Tables S3). Only those triplicate samples with best correlation coefficient (Fig. S1–3) were used to determine the differential protein abundances (Table S3). Ten proteins were observed down-regulated in the sticky diseased latex, 9 cysteine proteases (chymopapain) and 1 latex serine proteinase inhibitor (Table 2).

3.3 Proteolytic activity of papaya latex

In order to corroborate the differential proteome results, the proteolytic activity of crude PMeV-infected and healthy control latex (collected in the absence of protease inhibitors) was compared using casein as a substrate. At pH 8.0, the diseased latex presented a reduced proteolytic activity when compared with the healthy control latex (Fig. 3). The difference in activity was even more pronounced after latex treatment with DTT, a well-known activator of papaya latex cysteine proteases [2, 4]. E-64, an irreversible and specific cysteine protease inhibitor, known to inhibit chymopapain, papain, and caricain [35] was used to confirm that cysteine proteases of papaya latex were responsible for the differential proteolytic activity observed between diseased and healthy samples. In fact, the proteolytic activity was reduced to basal levels after the simultaneous treatment of the samples with DTT and E-64. This response pattern was also observed when the reaction was alternatively conducted in acidic condition (pH 6.5 and 5.0, Fig. 3) which more closely mimic the physiological pH of *C. papaya* latex [4]. Therefore, the sticky diseased papaya latex showed reduced proteolytic activity of cysteine proteases.

4. Discussions

The majority of the new *C. papaya* latex proteins found in the current study were associated with different types of stress (*e.g.*, cadmium ion, programmed cell death, drought, salinity, and microorganisms) suggesting that laticifers, besides their protective role against herbivorous insects [1, 3, 4] and wounding [4, 36], might be involved in other stress responses of the plant. In this context, the papaya sticky disease is a unique model to access the laticifers response against viral infection.

PMeV has been proposed to use the articulated and anastomosing laticifers of papaya as a network to move throughout the plant tissues[5, 6, 8]. Instead of moving, however, the content of this type of laticifers is expected to be in equilibrium, unless the laticifers are tapped. The positive turgor pressure would then drive the latex toward the wounded tissue [37]. Somehow, the exudation of latex is spontaneous in sticky diseased papaya [5–7]. The accumulation of potassium and phosphate in infected latex may drive water from the surrounding cells to the laticifers,[8] possibly accounting for the watery feature of the infected latex [5–8]. Here, osmotin was identified in papaya latex. The protein has been shown to be responsive to osmotic stress [38]. Two isoform of pectinesterase were also found in papaya latex suggesting that some cell-wall remodeling process could account for the laticifer's collapse. The enzyme was shown to be the host receptor for *Tobacco mosaic virus* (TMV) movement protein [39] and to enhance the RNA silencing against viral infection [40].

Since the PMeV infection of papaya laticifers is associated with the exudation of latex, the virus must cope with the proteases known to be activated during this event [16]. In fact, several proteases (31 cysteine-, 2 aspartic- and 1 C56-proteases) were observed in the latex of papaya, in which proteolysis was one of the most representative processes. Here, serine protease inhibitor, a protein known to be responsive to stress in plants [41], was shown down-regulated in papaya sticky diseased latex. Interestingly, 9 cysteine proteases were also significantly down-regulated in the sticky diseased papaya latex indicating that these

proteins are important in the plant response to PMeV. This reduction may have an inhibitory effect on the latex coagulation facilitating its flow through the laticifers and, consequently, the virus spread within the plant. Alternatively, the latex cysteine proteases might be involved with the laticifers' HR/PCD.

Proteases are important players of PCD in eukaryotes, which is essential in many aspects of animal and plant developmental processes and in response to various stresses [42]. Apoptosis, a form of animal PCD, involves cysteine proteases called caspases that cleave a limited set of cellular protein substrates [13]. In plants, caspase-like activities are also induced during HR and are inhibited by caspase inhibitors. Nevertheless, protease inhibitors such as E64, AEBSF, and TLCK, which do not inhibit plant caspase activities, can still block PCD, suggesting that additional proteases such as papain-like proteases are effectors or regulators of plant PCD [14]. Using protease inhibitors and virus-induced gene silencing, a papain cysteine protease, cathepsin B, was proven to be required for the disease resistance HR in plants [15]. In this study, several papaya latex cysteine proteases were predicted to be involved in developmental PCD. Thus, the modulation of cysteine proteases' levels, together with increased levels of H_2O_2 [8] might compose the HR of papaya laticifers to counteract the viral infection. Indeed, cysteine proteases and trypsin inhibitors were shown to be part of the pathogenesis-related proteins concomitantly accumulated with HR induction [17, 43].

The reduced cysteine protease content of papaya latex is actually reflected in the diseased latex, in which the cysteine-protease activity was considerably reduced. Additionally, the accumulation of H_2O_2 in the laticifers of infected papaya [8] might play a negative regulatory role on the cysteine-protease activity during the sticky disease [2, 4] probably by oxidizing and inactivating the active site of the enzyme. Taken together, these results indicate the negative modulation of cysteine proteases of papaya latex by PMeV infection, perhaps in attempt to delay the process of PCD in the laticifers [44]. In accordance, some fungal pathogens have been described to repress proteases from their hosts, for instance, an apoplastic papain-like protease from *Lycopersicon esculentum*, RCR3, which is a putative virulence target of the protease inhibitor, AVR2, from the fungal pathogen *Cladosporum fulvum* [45]. Furthermore, a cystatin (EPIC2) from *Phytophthora infestans* interacts with and inhibits PIP1, a novel papain-like cysteine protease [46].

Another notable feature of papaya latex proteome is the number of HSP70 isoforms identified. Eleven HSP70s were first reported here as occurring in the latex of papaya. HSP expression is frequently induced in response to both plant and animal viral infections [47]. The HSP70s may contribute to the folding and turnover of viral proteins as well as facilitate the viral movement [48]. If papaya HSP70s are favorable to PMeV infection, such an effect would be more likely correlated to laticifers since the proteomic analysis of sticky diseased papaya leaf tissues showed the down-regulation of the protein [10]. Indeed, up to now the virus was not found in papaya cells other than laticifers [5, 7]. Alternatively, HSP70s may play a role in the development of plant disease, perhaps protecting the cells from premature apoptosis [47]. Thus, the silencing of *Nicotiana benthamiana* HSP70 gene, NbHSP70c-1, abolished the hypersensitive response development after infiltration with the *P. infestans* protein INF1 or *Pseudomonas cichorii*, which normally triggers HR in the plant [49]. We propose that the HSP70s together with the reduction of the latex proteolysis might delay the PCD process developed in the laticifers against PMeV infection.

In this study, PMeV viral proteins homolog to those included in our database were not identified. This result might be related to both protein preparation method and the specific sample features. After resuspending the lyophilized latex in the aqueous buffer (100 mM Tris-HCl pH 7.6, 100 μM E-64), only part of the proteins could be solubilized and a pellet of latex was easily observed after sample centrifugation. The pellet was then submitted to

nucleic acid extraction using organic solvents and the PMeV 12 kbp dsRNA could be observed (data not shown). This confirmed that PMeV particles remained intact and linked to the solid phase of papaya latex, as described before [8]. This fact could explain why PMeV structural proteins were not identified. Moreover, high levels of proteolytic activity was observed for the sticky diseased latex, and some proteolytic activity remained even after E-64 treatment. Thus, less abundant proteins from papaya latex, and non-structural PMeV proteins might be degraded reducing their chances of identification.

5. Concluding remarks

The papaya latex has been known for years mostly as a source of papain and other cysteine proteases. The proteomic analysis conducted in this study added 122 newly identified proteins to the latex of *C. papaya*. A number of these proteins were predicted to be involved in plant stresses responses. Moreover, most of them could render protective roles to the laticifers, in addition to the known herbivorous toxicity and tissue protection after mechanical wounding. Notably, the proteolytic activity of the latex was severely reduced as a result of the lower amount of cysteine-proteases (chymopapain). Therefore, the changes at proteomic level described here, together with the known increment in laticifers-associated H_2O_2 production and the modulation of calcium levels in the latex during PMeV infection, are signs of HR of papaya laticifers against virus infection.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Ana Lúcia O. Carvalho (UFRJ) and João Piccin (UFES) for their helpful assistance. This work was supported by Financiadora de Estudos e Projetos (FINEP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Banco do Nordeste do Brasil (BNB), Fundação de Amparo à Pesquisa do Estado do Espírito Santo (FAPES) and Rio de Janeiro (FAPERJ). The authors thank the Biomolecule Analysis Core Facility/ BBRC/UTEP, supported by NIH grants 2G12RR008124-16A1 and 2G12RR008124-16A1S1 for the access to the LC-MS instrument.

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Highlights

We conducted the first proteomic analysis of *Carica papaya* latex and determined their differential abundance during sticky disease.

160 unique proteins were identified, 122 are new for the latex of papaya.

Cysteine proteases were down-regulated in PMeV infected latex.

These results are important in the understanding of plant laticifers responses against pathogen infection.

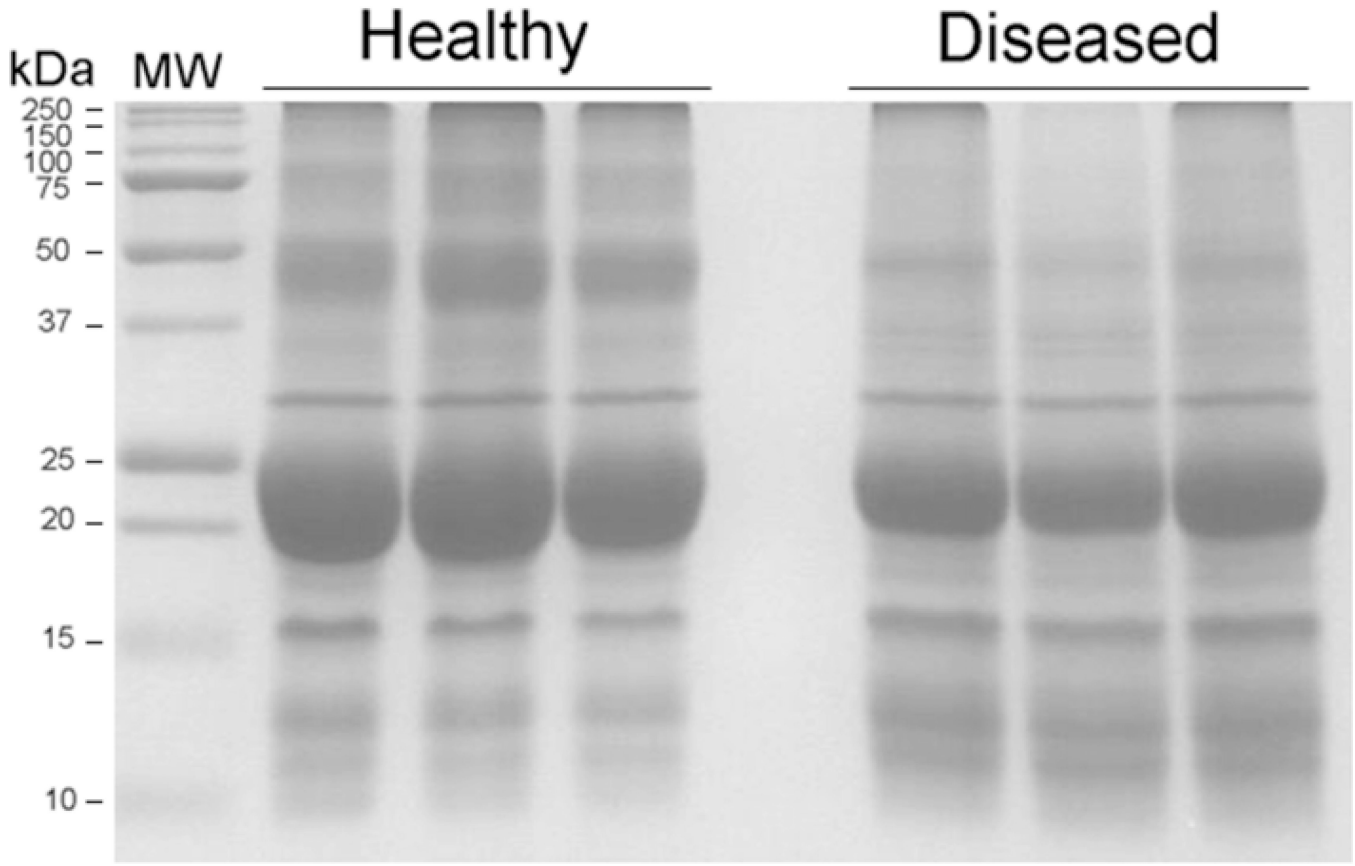

Fig. 1.

1-DE gel of the papaya latex proteins. Proteins (40 μg) from healthy and diseased latex previously solubilized in 100 mM Tris-HCl pH 7.6, containing 100 μM E-64, were separated using 12.5% SDS-PAGE. Gels were stained with colloidal CBB G-250. MW, molecular weight marker.

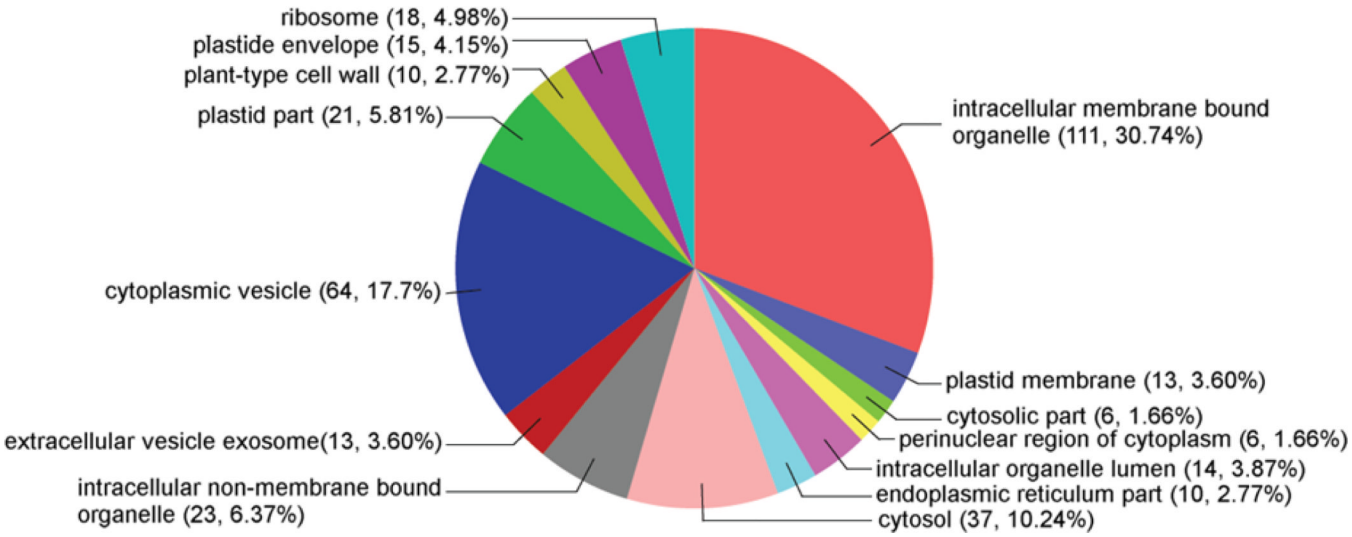

Fig. 2.

Gene ontology (GO) grouping of papaya latex proteins according to their associated cellular component using Blast2GO software. The numbers indicate the amount of sequences grouped in each GO term(s).

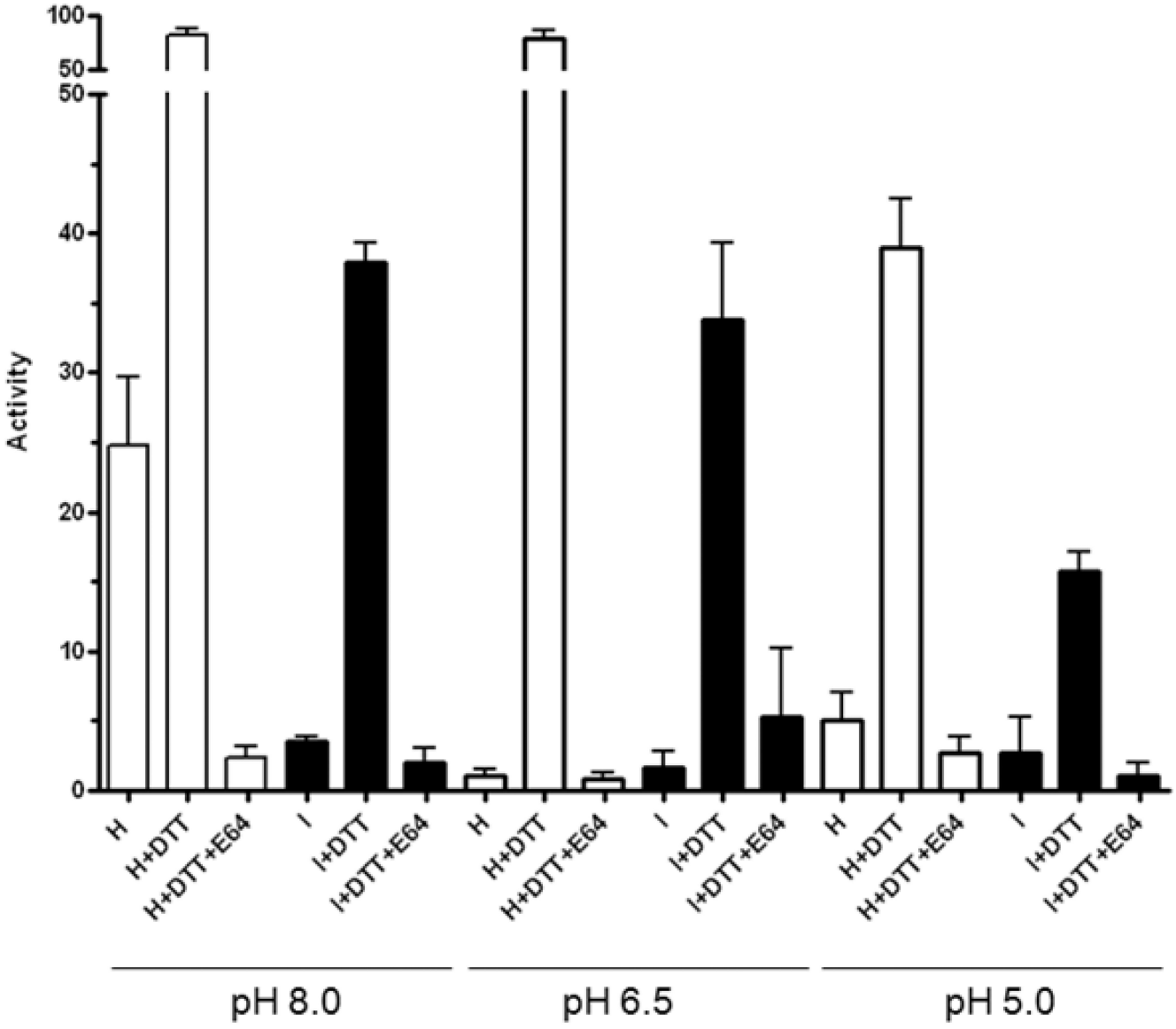

Fig. 3.

Proteolytic activity of *C. papaya* latex against casein substrate. Fifteen micrograms of lyophilized healthy (H) and infected (I) papaya latex were resuspended in Tris-HCl pH 8.0, phosphate buffer pH 6.5 or acetate pH 5.0, before incubation (37 °C for 30 min) with 1% (w/v) casein. Alternatively, 100 μM DTT or 5 μM E-64 was added to the reaction mixture. The protease activity was measured at 440 nm and expressed as the amount of enzyme that produced an increase of 0.001 unit of absorbance at $A_{440\ nm}$ per 30 min.

Table 1

Summary of the novel papaya latex proteins identified in this work.[a]

Protein group	Protein Name
19, 20, 29, 31, 43, 54, 66, 74, 87, 101, 173	Heat shock protein 70
28	Heat shock protein putative
126, 183	17.7 kDa heat shock protein
182	17.5 kDa class I heat shock protein
164	Putative heat shock protein 18
178	22.0 kDa class IV heat shock protein
11	Osmotin, putative
21	Methionine synthase
88	Cobalamin-independent methionine synthase isoz.
23, 108	Pectinesterase
24, 38	Pseudo-hevein (PR4)
45, 60, 130	Rubber peroxidase 1
51	Peroxidase C3, putative
47, 187	Annexin-like protein RJ4
49, 56, 82, 171	Protein disulfide-isomerase
147, 148, 188	Peptidyl-prolyl *cis-trans* isomerase
61, 135	Triacylglycerol lipase, putative
62, 90, 176	Enolase 1
68, 69, 81, 99	Glyceraldehyde-3-phosphate dehydrogenase
76	Calreticulin
89, 116	Ef-hand calcium binding protein, putative
107, 163, 181	Calcium ion binding protein, putative
133	Calmodulin
80, 149	Pore-forming toxin-like protein Hfr-2
84	Nuclear transport factor, putative
86	Adenosylhomocysteinase
94	Sodium/calcium exchanger protein
96, 110	Fructose-bisphosphate aldolase
98	Late embryogenesis abundant protein Lea14-A
102	Adenosine kinase
105, 168, 169	UDP-glucose pyrophosphorylase
106	Luminal binding protein
111, 143	Aspartyl aminopeptidase, putative
159	Protease C56, putative
112, 190	Serpin-like protein
113, 119, 131, 170	Phospholipase D alpha 1
115, 186	Agglutinin

Protein group	Protein Name
118	Triosephosphate isomerase
121	Actin-4
191	Tubulin alpha-6 chain
123	Phosphatidylglycerol/PI transfer protein, putative
128	Glutathione *S*-transferase
158, 140	Lactoylglutathione lyase
129	Major latex-like protein
136, 138	Putative polyubiquitin
151	UBQ8; protein binding
145	Beta-*N*-acetylhexosaminidase-like protein
146	Mitochondrial benzaldehyde dehydrogenase
150	Fructokinase, putative
152	Alpha-galactosidase-like protein
154	Aldo-keto reductase, putative
155	Lysosomal alpha-mannosidase, putative
156	Putative 60S ribosomal protein
160	Dihydroneopterin aldolase
161	Pathogenicity protein PATH531-like protein
165	Mutator-like transposase
166	Aquaporin, MIP family, TIP subfamily
172	Beta-*N*-acetylhexosaminidase-like protein
174	Coatomer gamma subunit, putative
179	Putative palmitoyl-protein thioesterase
180	*S*-adenosylmethionine synthase
184	Oxidoreductase, putative
185	Elongation factor 1-beta, putative
192	Disease resistance-like protein
13, 30	Unnamed protein product
122, 162, 167	Putative uncharacterized protein
141, 142, 177, 189	Unknown protein

[a]Detailed protein identification information is provided in Table S2.

Table 2

Down regulated protein in papaya sticky diseased latex

protein group	Protein name	Fold change (Log2 Infected/Healthy)	FDR
5	Chymopapain isoform II	−0.347	0.029826
7	Chymopapain isoform IV	−0.349	0.0221
8	Chymopapain isoform III	−0.349	0.0221
14	Latex serine proteinase inhibitor	−0.364	0.074907
26	Chymopapain isoform IV	−0.348	0.024175
33	Chymopapain (EC 3.4.22.6)	−0.346	0.030411
37	Chymopapain (EC 3.4.22.6)	−0.349	0.02203
40	Chymopapain (EC 3.4.22.6)	−0.326	0.084482
44	Chymopapain (EC 3.4.22.6)	−0.349	0.021985
55	Chymopapain (EC 3.4.22.6)	−0.341	0.047554