ORIGINAL ARTICLE

Extended latex proteome analysis deciphers additional roles of the lettuce laticifer

Won Kyong Cho · Xiong-Yan Chen ·
Yeonggil Rim · Hyosub Chu · Yeonhwa Jo ·
Suwha Kim · Zee-Yong Park · Jae-Yean Kim

Abstract Lettuce is an economically important leafy vegetable that accumulates a milk-like sap called latex in the laticifer. Previously, we conducted a large-scale lettuce latex proteomic analysis. However, the identified proteins were obtained only from lettuce ESTs and proteins deposited in NCBI databases. To extend the number of known latex proteins, we carried out an analysis identifying 302 additional proteins that were matched to the NCBI non-redundant protein database. Interestingly, the newly identified proteins were not recovered from lettuce EST and protein databases, indicating the usefulness of this hetero system in MudPIT analysis. Gene ontology studies revealed that the newly identified latex proteins are involved in many processes, including many metabolic pathways, binding functions, stress responses, developmental processes, protein metabolism, transport and signal transduction. Application of the non-redundant plant protein database led to the identification of an increased number of latex proteins. These newly identified latex proteins provide a rich source of information for laticifer research.

Keywords Lettuce · Latex · Laticifer · Proteomics

W. K. Cho and X.-Y. Chen contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s11816-010-0149-9) contains supplementary material, which is available to authorized users.

W. K. Cho · X.-Y. Chen · Y. Rim · H. Chu · Y. Jo ·
J.-Y. Kim (✉)
Division of Applied Life Science (BK21 Program),
Environmental Biotechnology, National Core Research Center,
PMBBRC, Gyeongsang National University,
Jinju 660-701, Republic of Korea
e-mail: kimjy@gnu.ac.kr

S. Kim · Z.-Y. Park
Department of Life Science,
Gwangju Institute of Science and Technology,
Gwangju 500-712, Republic of Korea

Introduction

Higher plants have the ability to transport nutrients, water, and numerous molecules from source to sink via a vascular system composed of phloem and xylem (Carlsbecker and Helariutta 2005). In addition to these two representative tissue types, a restricted number of plant families contain laticifers, which are distinguishable conduit systems that produce a milk-like sap called latex (Pickard 2008). It has been reported that more than 20 plant families produce latex, such as the Asteraceae family, the poppy family, greater celandines, and rubber trees (Hagel et al. 2008).

Laticifers are classified not by their contents but by their morphological characteristics and developmental patterns (Hagel et al. 2008). Furthermore, laticifers consist of multinuclear cells that provide tissue specificity. Some latex-containing plants are very commercially useful. For example, natural rubber is produced in the latex of the rubber tree and is synthesized within laticifers of the opium poppy (Chow et al. 2007; Decker et al. 2000; Nawrot et al. 2007). It has been suggested that the laticifer plays an important role as the point of deposition of various metabolites and defense mechanisms against pathogens (Hagel et al. 2008).

However, evidence from previous studies could not explain such hypotheses. Therefore, we selected lettuce, which is an ideal plant to sample a large amount of latex sap from stems and leaves. Using multidimensional protein-identification technology (MudPIT), we previously

identified 587 lettuce latex proteins obtained only from lettuce ESTs and proteins (Cho et al. 2009). However, the limited number of lettuce genomic sequences made proteomic analysis difficult. A recent study leading to the identification of more than one thousand pumpkin phloem proteins also reported the successful application of different databases, including the pumpkin EST database and non-redundant plant protein database (Lin et al. 2009).

To extend the number of known latex proteins, we made use of previous peptide information for the additional identification of latex proteins from the NCBI non-redundant protein database. Interestingly, the newly identified proteins, for the most part, were not recovered from lettuce EST and protein databases, indicating the usefulness of this hetero system in MudPIT analysis. Furthermore, bioinformatic analyses revealed that many important biological processes occur in the lettuce laticifer.

Materials and methods

General experimental procedures

The lettuce latex sampling, SDS-PAGE gels, in-gel digestion and peptide sample preparation, and micro-LC/LC-MS/MS analytical procedures utilized in this study have been described previously (Cho et al. 2009).

Database search

All MS/MS spectra obtained from LC-MS/MS analyses were searched against a database containing the non-redundant plant protein database downloaded from NCBI (http://www.ncbi.nlm.nih.gov/). Bioworks (v.3.1) was used to filter the search results, and the following Xcorr values and a delta Cn value of 0.08 were applied to the different charge states of peptides: 1.8 for singly charged peptides, 2.5 for doubly charged peptides, and 3.5 for triply charged peptides. Peptide and protein identification were validated using the Scaffold program (version Scaffold-01_07_00, Proteome Software Inc., Portland, OR, USA) with default parameters. Peptides were identified based on more than 95% probability, and proteins were accepted with more than 99% probability and at least two unique peptide matches.

BLAST search and gene ontology analysis

A standalone BLAST program was downloaded from NCBI and installed in a Linux system. A total of 302 latex proteins were BLASTed against Arabidopsis proteome data obtained from TAIR (http://www.arabidopsis.org/, version TAIR8) (Rhee et al. 2003). BLASTP and 1e-30 were used

to determine the BLAST algorithm and e value, respectively. Gene ontology (GO) analyses were performed using the GO annotation search tool within the TAIR website. To obtain detailed information regarding specific cellular processes, 245 non-redundant Arabidopsis protein sequences that were matched to 302 latex proteins were applied to the Blast2GO program using default parameters.

Results

Identification of additional proteins present in lettuce latex using the non-redundant plant protein dataset

The comprehensive lettuce latex proteome was investigated previously through LC-MS/MS coupled with enzyme digestion, revealing a total number of 587 novel proteins that are present in lettuce latex sap (Cho et al. 2009). The identified proteins were only derived from lettuce EST and lettuce protein databases that were downloaded from NCBI. However, the number of identified proteins was still lower than that seen in a recent large-scale pumpkin phloem proteome analysis, and latex protein functions were also restricted to specific processes. Therefore, it would be meaningful to extend the number of identified proteins based on protein homology to other plant species using the non-redundant plant database. It has been proposed that such an approach does not always provide correct data; however, it is a useful approach when complete genome sequences are not available, such as in the recent pumpkin phloem proteome analysis (Habermann et al. 2004; Lin et al. 2009).

All MS/MS spectra obtained from the previous study were used to search for sequence similarities within the NCBI non-redundant plant protein database. A total of 302 latex proteins were newly identified after applying at least two peptide matches. The majority of the proteins were highly homologous to those of Arabidopsis. The full list of the 302 newly identified latex proteins and further peptide information can be found in Tables 1 and 2 of the "Electronic supplementary material," respectively.

Representative latex proteins are listed in Table 1. They were selected with high sequence coverage (more than 20%). For example, several proteins involving ubiquitin-mediated proteolysis were identified, such as ubiquitin-fold modifier 1, ubiquitin, ubiquitin-activating enzyme, and ubiquitin extension protein. Moreover, a glyceraldehyde-3-phosphate dehydrogenase C subunit, vacuolar ATP synthase subunit B, H+-transporting ATPase, phosphoglycerate kinase, and pyruvate kinase are also abundantly present in lettuce latex.

We were interested in testing how many new proteins were identified by applying the non-redundant protein database for protein identification. To compare two

Table 1 Representative proteins present in lettuce latex sap

GI accession no.	Descriptive name	Sequence count	Sequence coverage (%)	Molecular mass (Da)	pI
12323301	Ubiquitin-fold modifier 1 precursor	2	54	9929	9.2
28202244	Ubiquitin	6	45	8525	7.2
29294049	Calmodulin-6 (CAM6)	5	41	16834	4.3
30039180	Copper chaperone	3	40	8547	6.6
95116512	Ubiquitin activating enzyme	2	38	11163	5
6721173	Glyceraldehyde-3-phosphate dehydrogenase C subunit (GapC)	14	37	36914	7.1
1765896	Rab2-like protein	8	35	23164	7.4
6721109	Vacuolar ATP synthase subunit B	12	35	58166	5.2
7270834	Probable H$^+$-transporting ATPase	11	34	54305	5.1
8778573	Ubiquitin extension protein, 40S ribosomal protein S27A (RPS27aA)	4	32	17672	9.8
8778579	A member of ARF GTPase family	4	31	28397	8.9
12321751	GTP-binding protein (SAR1B)	7	30	21986	7
62321423	Putative glyceraldehyde-3-phosphate dehydrogenase	3	27	17309	8.3
62319057	Phosphoglycerate kinase-like protein	2	27	13339	4.6
83754659	GDP-mannose-3′,5′-epimerase	6	25	42830	6.1
12321760	Phosphoglycerate kinase	14	23	49939	8.3
8052544	G-box binding factor GF14 omega	5	22	29162	4.8
23397188	Pyruvate kinase	11	21	57495	7.1
7767657	26S proteasome AAA-ATPase subunit RPT4a-like protein	5	20	44756	8.2

different protein datasets, it is necessary to convert each latex protein into the corresponding *Arabidopsis* accession number. The 302 newly identified proteins, as well as 559 other proteins, were BLASTed against the *Arabidopsis* proteome using an *e* value of $1e - 30$ as a cutoff. The previous study also reported that many viral proteins were present in latex. Therefore, we excluded 28 viral proteins from the list of latex proteins. After the BLAST search, all 302 newly identified latex proteins were highly matched to the *Arabidopsis* proteome; however, only 455 proteins from the previous study had significant homology to *Arabidopsis*, suggesting that the remaining 104 proteins could be lettuce-specific latex proteins. Finally, we obtained 245 and 379 non-redundant *Arabidopsis* accession numbers from this and our previous study, respectively. Surprisingly, after the two datasets were compared, only 12 proteins were found to be common to both, as shown in Fig. 1. This result strongly suggests that multiple databases can be successfully applied to protein identification when the full genome sequence of a given plant species is not available.

Distribution of molecular mass and isoelectric points

The 302 newly identified latex proteins have a wide range of molecular masses (see Fig. 2). For example, 20 proteins have molecular masses of less than 20 kDa, indicating the presence of small molecules in lettuce latex. We found 10
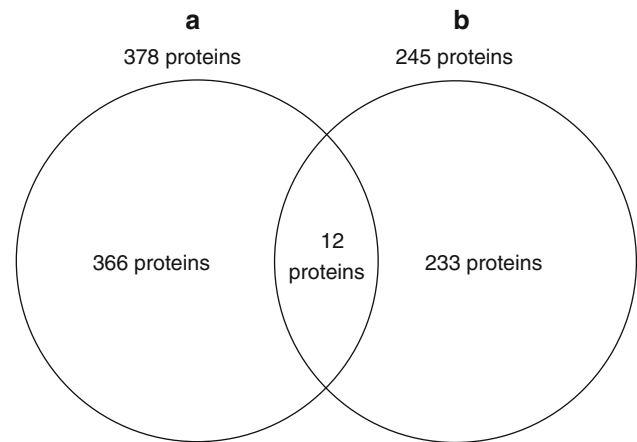

Fig. 1 Comparison of the number of identified latex proteins in this study versus a previous study. To compare the two datasets, the identified lettuce proteins were converted to the corresponding *Arabidopsis* accession numbers. Non-redundant *Arabidopsis* accession numbers were used in each study. **a** Proteins identified in the previous study (Cho et al. 2009), and **b** proteins identified in this study

proteins with molecular masses greater than 130 kDa. Interestingly, the molecular mass distribution of latex proteins in this study was very different from that of a previous study, which identified 587 latex proteins that—for the most part—had molecular masses of between 20 and 30 kDa. This difference could be caused by differences
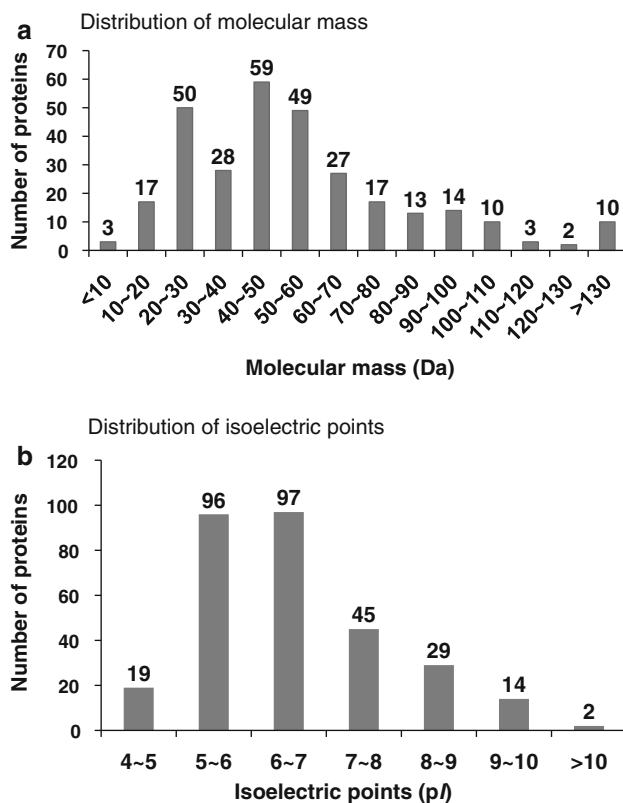
Fig. 2 The distribution of molecular masses (**a**) and isoelectric points (**b**) of 302 newly identified latex proteins

in sequence lengths between EST and non-redundant plant protein databases. For instance, EST sequence lengths are generally shorter than full-length sequences. In addition, the newly identified proteins in this study belong to a wider range of biological processes than those found in the previous study, including a large number of resistance gene families.

The 302 newly identified latex proteins have a wide range of isoelectric points, from 4.3 to 11. The majority of the proteins (64%) have isoelectric points (pI values) that range from 5 to 7. In addition, 19 proteins (p$I < 5$) are acidic and 16 proteins (p$I > 9$) are very alkaline.

Gene ontology analysis of newly identified lettuce latex proteins

To obtain insight into the functions of the 302 newly identified latex proteins, we used the *Arabidopsis* proteome, in which annotations are well characterized. The 302 proteins were converted to the corresponding *Arabidopsis* accession numbers, as described in "Materials and methods." Finally, 245 non-redundant *Arabidopsis* proteins were subjected to a GO annotation search. Proteins were assigned to 894 GO terms (41%) for cellular components, 922 (43%) for

biological processes, and 343 (16%) for molecular functions (Fig. 3a).

GO cellular components

"Other intracellular component" ($n = 186$) was the most common GO term among the cellular components, followed by "other cytoplasmic component" ($n = 138$). "Chloroplast-targeted proteins" ($n = 115$) and "plasma membrane proteins" ($n = 94$) were the third and fourth most common terms and were highly enriched in latex proteins. Interestingly, as shown in the previous latex proteomic analysis, the GO analysis provides strong evidence that latex contains a large number of proteins that are localized in chloroplasts ($n = 115$) and mitochondria ($n = 34$). In addition, cell wall ($n = 33$) and extracellular ($n = 29$) components revealed that many secretory proteins may be retained in latex. Some latex proteins may be localized in the ribosome ($n = 16$), Golgi apparatus ($n = 5$), and endoplasmic reticulum ($n = 4$), and these data suggest that protein synthesis and vesicular transport are also likely to occur in laticifers. To validate the cellular component results, the subcellular localizations of the 302 newly identified lettuce latex proteins were predicted using TargetP. A large number of proteins (72%) were assigned to unknown subcellular localizations (Fig. 4). Of the proteins with predicted subcellular localizations, chloroplasts (14%), mitochondria (6%), and secretory pathway proteins (8%) were the most abundant in latex (Fig. 4).

GO biological processes

Analysis of the GO biological processes of latex proteins provides evidence that several cellular ($n = 220$) and metabolic ($n = 205$) processes are present in latex (Fig. 3c). GO terms related to stress response ($n = 96$) are highly enriched. Many of these proteins probably respond to abiotic and biotic stimuli ($n = 92$). GO terms related to metabolic proteins ($n = 88$) are also highly enriched. Latex proteins could play important roles in developmental processes ($n = 37$). They also participate in the transport of macromolecules ($n = 36$) within the laticifer, which is a route for transporting electrons and energy ($n = 24$). Furthermore, the laticifer is the place where signal transduction ($n = 15$), cell organization and biogenesis ($n = 12$), and transcription ($n = 12$) also occur.

GO molecular functions

GO terms relating to molecular function suggest that numerous enzyme activities and binding functions are present in the laticifer (Fig. 3d). For example, proteins related to other enzyme activities ($n = 82$), transferase
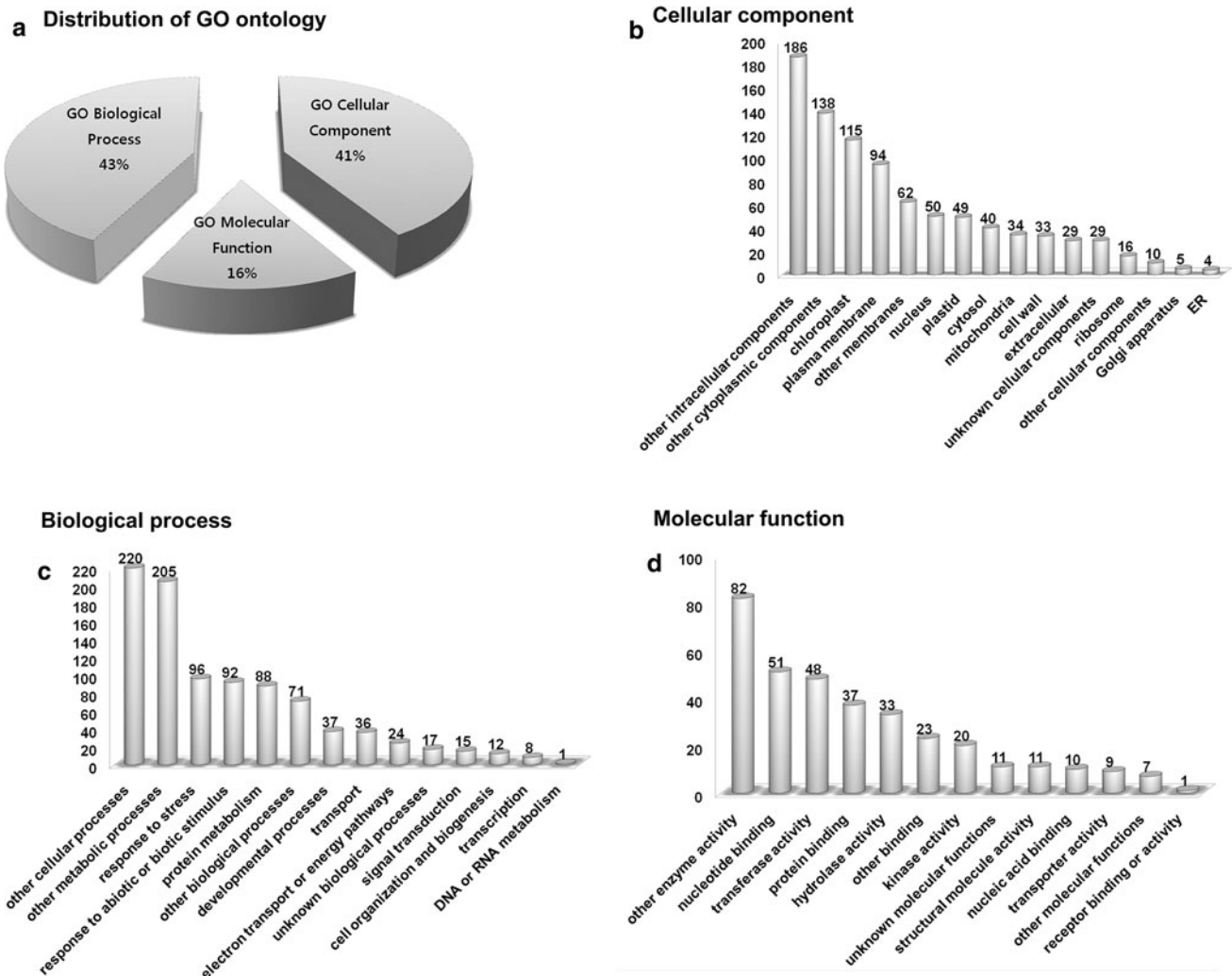
a **Distribution of GO ontology**

b **Cellular component**

Biological process

Molecular function

Fig. 3 Gene ontology analysis of newly identified lettuce latex proteins. To gain insight into the functional distribution of the 302 newly identified latex proteins, 245 homologous, non-redundant *Arabidopsis* accession numbers were used for annotation. **a** Distribution of gene ontology; **b** GO frequency by cellular component; **c** GO frequency by biological process; **d** GO frequency by molecular function
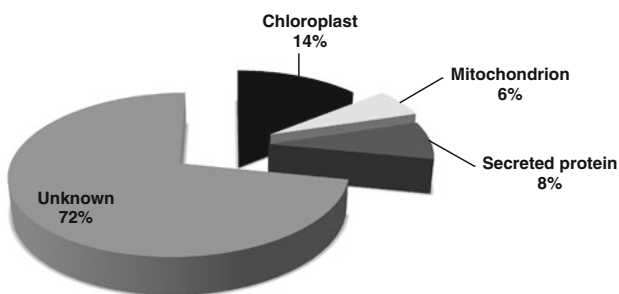

Fig. 4 Distribution of the subcellular localizations of newly identified lettuce latex proteins, as predicted by the TargetP program

activity ($n = 48$), hydrolase activity ($n = 33$), and kinase activity ($n = 20$) are highly enriched. Several proteins play critical roles by having binding functions to nucleotides

($n = 51$), proteins ($n = 37$), and nucleic acids ($n = 10$). Structural molecule activity ($n = 11$) and transporter activity ($n = 9$) are also detected.

Functional classification of latex proteins according to FunCat annotations

A subset of 302 lettuce proteins was assigned functional classification according to *Arabidopsis* functional annotations in FunCat (Table 2). Among them, only 14 proteins were assigned as unclassified proteins. Proteins involving metabolism (46%) and proteins with binding functions (46.9%) fell into the largest functional groups. The metabolic pathways present in latex are amino acid, nitrogen, sulfur, nucleotide, phosphate, carbohydrate, fatty acid, vitamin, and secondary product metabolism. The latex

Table 2 Functional categories of 245 *Arabidopsis* proteins according to FunCat annotations

Functional category	No. of latex proteins	*Arabidopsis* genome	*P* value
Metabolism	112 (46.0%)	4932 (17.3%)	2.37×10^{-25}
Energy	35 (14.4%)	453 (1.59%)	3.73×10^{-23}
Cell cycle and DNA processing	6 (2.46%)	1518 (5.34%)	99.1
Transcription	9 (3.70%)	2664 (9.37%)	1.00
Protein synthesis	16 (6.58%)	1332 (4.68%)	0.109
Protein fate (folding, modification, destination)	68 (27.9%)	3104 (10.9%)	1.44×10^{-13}
Protein with binding function or cofactor requirement (structural or catalytic)	114 (46.9%)	7027 (24.7%)	4.46×10^{-14}
Regulation of metabolism and protein function	6 (2.46%)	608 (2.13%)	0.420
Cellular transport, transport facilities and transport routes	30 (12.3%)	2419 (8.51%)	2.54×10^{-2}
Cellular communication/signal transduction mechanism	15 (6.17%)	1283 (4.51%)	0.138
Cell rescue, defense and virulence	41 (16.8%)	1425 (5.01%)	8.37×10^{-12}
Interaction with the environment	34 (13.9%)	1651 (5.81%)	1.90×10^{-6}
Systemic interaction with the environment	7 (2.88%)	757 (2.66%)	0.471
Cell fate	4 (1.64%)	450 (1.58%)	0.539
Development (systemic)	12 (4.93%)	1047 (3.68%)	0.188
Biogenesis of cellular components	10 (4.11%)	1554 (5.46%)	0.861
Subcellular localization	136 (55.9%)	10531 (37.0%)	1.45×10^{-9}
Organ localization	1 (0.41%)	42 (0.14%)	0.303
Unclassified proteins	14 (5.76%)	7994 (28.1%)	1.00

proteins converted to *Arabidopsis* accession numbers involving several metabolic pathways, as well as their respective enzyme commission (EC) numbers, are listed in Table 3 of the "Electronic supplementary material." At least 10 EC numbers for glycolysis were identified. In addition, it seems that plant hormones are synthesized within laticifers, considering the identification of 17 EC numbers that are required for plant hormone biosynthesis. Also found were 14 EC numbers for biosynthesis of alkaloids derived from terpenoids and polyketides, 9 for purine metabolism, 4 for fructose and mannose metabolism, 6 for pyruvate metabolism, and 6 for pyruvate metabolism. These data provide strong evidence that latex sap contains numerous enzymes required for many important metabolic pathways. In addition, the presence of protein synthesis (6.58%) and protein folding, modification and destination (27.9%) indicate that protein regulation is an important function in latex sap (Table 2).

Conserved domains in latex proteins

We were interested in finding conserved domains within latex proteins in order to gain insight into latex protein families. Therefore, we conducted a conserved domain analysis using the InterPro program. A total of 2733 InterPro domains were identified. The most frequently matched domains are listed in Table 3. NAD(P)-binding, chaperonin Cpn60/TCP-1, ATPase, Ras small GTPase, 26S proteasome subunit P45, 20S proteasome A and B subunits, proteasome alpha-subunit, ubiquitin, heat shock protein Hsp70, and Ras GTPase are the ten most common domains present in latex proteins.

Discussion

Successful application to expand the scope of the latex proteome by cross-species protein identification

The main purpose of this study was to find additional latex proteins using the non-redundant plant database. Successful application of a homology-based approach led to the identification of a total of 302 latex proteins. Surprisingly, a comparison study of the two datasets revealed that they had only 12 proteins in common, demonstrating the novelty of these newly identified latex proteins. The unavailability of a full genome sequence for an organism of study has always been an obstacle to proteomic analysis. However, previous reviews proposed that cross-species protein identification in mass spectrometry might facilitate the identification of proteins of organisms with poor sequence data from phylogenetically related organisms with significant sequence

Table 3 The 36 conserved domains most frequently found in latex proteins

InterPro	Description	No. of proteins
IPR016040	NAD(P)-binding	95
IPR002423	Chaperonin Cpn60/TCP-1	48
IPR003593	ATPase, AAA+ type, core	36
IPR003579	Ras small GTPase, Rab type	32
IPR005937	26S proteasome subunit P45	30
IPR001353	20S proteasome, A and B subunits	28
IPR000426	Proteasome alpha-subunit, conserved site	27
IPR000626	Ubiquitin	27
IPR001023	Heat shock protein Hsp70	27
IPR001806	Ras GTPase	27
IPR000308	14-3-3 protein	26
IPR005225	Small GTP-binding protein	26
IPR001395	Aldo/keto reductase	24
IPR001404	Heat shock protein Hsp90	24
IPR001697	Pyruvate kinase	24
IPR015793	Pyruvate kinase, barrel	24
IPR015794	Pyruvate kinase, alpha/beta	24
IPR016024	Armadillo-type fold	22
IPR006195	Aminoacyl-tRNA synthetase, class II, conserved region	21
IPR013785	Aldolase-type TIM barrel	21
IPR002133	*S*-adenosylmethionine synthetase	20
IPR003594	ATP-binding region, ATPase-like	20
IPR003959	ATPase, AAA-type, core	20
IPR004000	Actin/actin-like	20
IPR000741	Fructose-bisphosphate aldolase, class-I	18
IPR000941	Enolase	18
IPR001576	Phosphoglycerate kinase	18
IPR001650	DNA/RNA helicase, C-terminal	18
IPR013126	Heat shock protein 70	18
IPR013753	Ras	18
IPR001473	Clathrin, heavy chain, propeller, N-terminal	16
IPR004100	ATPase, F1/V1/A1 complex, alpha/beta subunit, N-terminal	16
IPR006689	ARF/SAR superfamily	16
IPR000173	Glyceraldehyde 3-phosphate dehydrogenase	15
IPR000608	Ubiquitin-conjugating enzyme, E2	15
IPR015813	Pyruvate/phosphoenolpyruvate kinase, catalytic core	15

similarity and full genome sequences (Habermann et al. 2004; Liska and Shevchenko 2003). For example, latex proteins in the opium poppy have largely been characterized from *Arabidopsis* (31 proteins) as well as other organisms (Decker et al. 2000). In addition, the phloem proteome analysis in *Brassica napus* sap relied solely on the genome sequences of *Arabidopsis*, which is a close relative (Giavalisco et al. 2006). In addition, a recent pumpkin phloem proteomic analysis identified more than 1000 proteins (Lin et al. 2009). Although a large number of pumpkin phloem proteins were identified from the pumpkin EST database, more than 500 proteins were identified from other plant species, such as *Arabidopsis*, rice, poplar, grape, etc. Therefore, our efforts to increase the number of identified proteins using the non-redundant plant protein database were successful, and the number of proteins from the combined datasets is sufficiently large to be used as a resource to study latex functions.

Stress response-related proteins

A total of 35 proteins were grouped into the stress response category. They are involved in oxidative stress, osmotic and salt stress, heat shock, cold shock, and nutrient

starvation. For example, an *Arabidopsis* homolog disease resistance protein (AT5G45200) and two heat shock proteins (AT5G52640 and AT5G56030) are known to be responsible for defense against biotic and abiotic stresses. Malate dehydrogenase (NAD), aspartate transaminase, phosphoglycerate mutase, chloroplast chaperonin 60, nuclear phosphoglycerate kinase (PGK1), chloroplast heat shock protein 70-1, enolase and calcium-independent ABA-activated protein kinase play roles in cold stress. The *Arabidopsis* mutants *los1* (AT1G56070) and *los2* (AT2G36530) inhibit cold-response gene transcription and are tolerant of freezing (Guo et al. 2002; Lee et al. 2002). Genetic studies have identified that the genes encoding translation elongation factor 2-like protein and enolase, respectively, were defective in these two mutants. These data provide a hypothesis that cold-induced transcriptional controls occur in latex sap.

Functions of GTP-binding proteins in latex sap

One striking feature of this analysis is that latex sap contains more than 10 proteins that belong to the group of GTP-binding proteins. GTP-binding proteins are activated by GTP and inactivated by the hydrolysis of GTP to GDP (Vernoud et al. 2003). They are involved in various cellular processes, including signal transduction, cell proliferation, cytoskeletal organization and intracellular membrane trafficking (Vernoud et al. 2003). GTP-binding proteins can be divided into several distinct families (Kahn et al. 1992). Most GTP-binding proteins in latex are Ras GTPases. In *Arabidopsis*, these proteins have various characterized functions. They are involved in plant development processes, including pollen tube growth, root hair development and cell expansion (Li et al. 2001; Yang 2002). In addition, they regulate H_2O_2 production and the ABA response (Yang 2002). However, the detailed functional roles of these proteins with regard to latex sap are not yet known.

Proteins required for the ubiquitin/proteasome pathway

Post-translational modification is one of most important processes involved in protein stabilization and activation. Of the known post-translational modifications, the ubiquitin/proteasome pathway plays critical roles in a broad range of cellular processes, such as hormone signaling, plant development, and plant–microbe interactions (Moon et al. 2004; Smalle and Vierstra 2004; Zeng et al. 2006). Recently, a pumpkin phloem proteomic analysis reported more than 100 proteins involved in ubiquitin-mediated proteolysis (Lin et al. 2009). Thus, we proposed that the functional roles of the laticifer might be quite similar to that of phloem, based on evidence that several components of the ubiquitin/26S proteasome system function in the protein degradation pathway in latex sap.

Metabolic proteins in lettuce latex sap

It is not surprising that latex sap contains enzymes involved in a wide range of metabolic pathways. More than 100 proteins in this study were enzymes involved in general metabolism. For example, several glycolysis enzymes were found in latex sap, including alcohol dehydrogenase, phosphopyruvate hydratase, pyruvate kinase, phosphoglycerate mutase, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase and triose-phosphate isomerase. In addition, fructose and mannose metabolism enzymes, such as fructose-bisphosphate aldolase and aldehyde reductase, were also detected in this study. Furthermore, it was found that enzymes involved in starch and sucrose metabolism, including UDP-glucose 6-dehydrogenase, UTP–glucose-1-phosphate uridylyltransferase, UDP-glucuronate decarboxylase and galacturan 1,4-alpha-galacturonidase, also occur in latex sap. These findings correlate highly with a previous study, which determined that general metabolism occurred in the latex of *Papaver somniferum* (Antoun and Roberts 1975). In addition, they play important roles in alkaloid biogenesis, which is a unique feature of latex-containing plants (Roberts et al. 1983). Such a strong presence of metabolic pathway enzymes was also reported in the pumpkin phloem proteome, suggesting common features between latex and phloem (Lin et al. 2009). However, the production of species-specific metabolites, such as lactucin, cardenolides, alkaloids and natural rubber, provides evidence that the laticifer is distinct from phloem (Hagel et al. 2008; Sessa et al. 2000).

So far, a large number of latex proteins from *Lactuca sativa*, *Papaver somniferum* and *Chelidonium majus* have been reported (Cho et al. 2009; Decker et al. 2000; Nawrot et al. 2007). Their roles are mostly focused on plant defense mechanisms against pathogens and insects. However, the newly identified latex proteins in this study provide evidence that latex sap proteins function in various biological processes.

References

Antoun MD, Roberts MF (1975) Some enzymes of general metabolism in the latex of *Papaver somniferum*. Phytochemistry 14:909–914

Carlsbecker A, Helariutta Y (2005) Phloem and xylem specification: pieces of the puzzle emerge. Curr Opin Plant Biol 8:512–517

Cho WK, Chen XY, Uddin NM, Rim Y, Moon J, Jung JH, Shi C, Chu H, Kim S, Kim SW, Park ZY, Kim JY (2009) Comprehensive proteome analysis of lettuce latex using multidimensional protein-identification technology. Phytochemistry 70:570–578

Chow KS, Wan KL, Isa MN, Bahari A, Tan SH, Harikrishna K, Yeang HY (2007) Insights into rubber biosynthesis from transcriptome analysis of *Hevea brasiliensis* latex. J Exp Bot 58:2429–2440

Decker G, Wanner G, Zenk MH, Lottspeich F (2000) Characterization of proteins in latex of the opium poppy (*Papaver somniferum*) using two-dimensional gel electrophoresis and microsequencing. Electrophoresis 21:3500–3516

Giavalisco P, Kapitza K, Kolasa A, Buhtz A, Kehr J (2006) Towards the proteome of *Brassica napus* phloem sap. Proteomics 6:896–909

Guo Y, Xiong L, Ishitani M, Zhu JK (2002) An *Arabidopsis* mutation in translation elongation factor 2 causes superinduction of CBF/DREB1 transcription factor genes but blocks the induction of their downstream targets under low temperatures. Proc Natl Acad Sci USA 99:7786–7791

Habermann B, Oegema J, Sunyaev S, Shevchenko A (2004) The power and the limitations of cross-species protein identification by mass spectrometry-driven sequence similarity searches. Mol Cell Proteomics 3:238–249

Hagel JM, Yeung EC, Facchini PJ (2008) Got milk? The secret life of laticifers. Trends Plant Sci 13:631–639

Kahn RA, Der CJ, Bokoch GM (1992) The ras superfamily of GTP-binding proteins: guidelines on nomenclature. FASEB J 6:2512–2513

Lee H, Guo Y, Ohta M, Xiong L, Stevenson B, Zhu JK (2002) LOS2, a genetic locus required for cold-responsive gene transcription encodes a bi-functional enolase. EMBO J 21:2692–2702

Li H, Shen JJ, Zheng ZL, Lin Y, Yang Z (2001) The Rop GTPase switch controls multiple developmental processes in *Arabidopsis*. Plant Physiol 126:670–684

Lin MK, Lee YJ, Lough TJ, Phinney BS, Lucas WJ (2009) Analysis of the pumpkin phloem proteome provides insights into angiosperm sieve tube function. Mol Cell Proteomics 8:343–356

Liska AJ, Shevchenko A (2003) Expanding the organismal scope of proteomics: cross-species protein identification by mass spectrometry and its implications. Proteomics 3:19–28

Moon J, Parry G, Estelle M (2004) The ubiquitin-proteasome pathway and plant development. Plant Cell 16:3181–3195

Nawrot R, Kalinowski A, Gozdzicka-Jozefiak A (2007) Proteomic analysis of *Chelidonium majus* milky sap using two-dimensional gel electrophoresis and tandem mass spectrometry. Phytochemistry 68:1612–1622

Pickard WF (2008) Laticifers and secretory ducts: two other tube systems in plants. New Phytol 177:877–888

Rhee SY, Beavis W, Berardini TZ, Chen G, Dixon D, Doyle A, Garcia-Hernandez M, Huala E, Lander G, Montoya M, Miller N, Mueller LA, Mundodi S, Reiser L, Tacklind J, Weems DC, Wu Y, Xu I, Yoo D, Yoon J, Zhang P (2003) The *Arabidopsis* information resource (TAIR): a model organism database providing a centralized, curated gateway to *Arabidopsis* biology, research materials and community. Nucleic Acids Res 31:224–228

Roberts MF, McCarthy D, Kutchan TM, Coscia CJ (1983) Localization of enzymes and alkaloidal metabolites in *Papaver latex*. Arch Biochem Biophys 222:599–609

Sessa RA, Bennett MH, Lewis MJ, Mansfield JW, Beale MH (2000) Metabolite profiling of sesquiterpene lactones from *Lactuca* species. Major latex components are novel oxalate and sulfate conjugates of lactucin and its derivatives. J Biol Chem 275:26877–26884

Smalle J, Vierstra RD (2004) The ubiquitin 26S proteasome proteolytic pathway. Annu Rev Plant Biol 55:555–590

Vernoud V, Horton AC, Yang Z, Nielsen E (2003) Analysis of the small GTPase gene superfamily of *Arabidopsis*. Plant Physiol 131:1191–1208

Yang Z (2002) Small GTPases: versatile signaling switches in plants. Plant Cell 14(Suppl):S375–S388

Zeng LR, Vega-Sanchez ME, Zhu T, Wang GL (2006) Ubiquitination-mediated protein degradation and modification: an emerging theme in plant–microbe interactions. Cell Res 16:413–426