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Original Article

Comparative proteomic analysis reveals changes in proteome of natural rubber latex in response to hormonal stimulation and plant maturation

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Abstract

Availability of *cis*-1,4-polyisoprene from *Hevea brasiliensis* has become important for many kinds of products, as it is used in automobiles, gloves, and adhesives. Increasing the yield of latex from rubber trees would contribute to income in tropical countries. The latex yield depends on various factors, including plant hormones and aging. This work focused on studying the effects of these factors on dry rubber content and on proteome profiles. The results suggest that dry rubber content slightly decreased upon hormonal treatment, and increased with maturation. Both factors affected the protein concentration. Comparative proteomic analysis indicated that ethylene stimulation altered abundances of 1,553 soluble proteins and 1,001 rubber-bound proteins. Plant maturation affected abundances of 2,961 soluble proteins and 839 rubber-bound proteins. These results provide useful information about the protein-level mechanisms of rubber biosynthesis, affecting rubber yield in response to hormone treatment and aging.

Keywords: rubber latex, *Hevea brasiliensis*, proteomics, ethylene, aging

1. Introduction

Rubber plants (*Hevea brasiliensis*) are economically important due to their natural rubber latex utilized in a wide variety of applications. The major component of the milky latex is a long-chained polymer of *cis*-1, 4-polyisoprene units, which possesses high elasticity and high tensile strength, providing beneficial material properties for applications in gloves, automotive parts, and medical devices, among others. The minor components include proteins, carbohydrates, phospholipids and inorganic minerals (Jacob, d'Auzac, & Prevot, 1993; Sansatsadeekul, Sakdapipanich, & Rojruthai, 2011). Fresh natural rubber latex is known to be generated in the cytoplasm of specialized cells, so-called laticifer cells, found in rubber-producing plants. The latex can be fractionated by centrifugation, resulting in three main layers, namely rubber particles, C-serum, and lutoid particles from top to bottom (Moir, 1959). Proteins in the rubber latex have been reported to be distributed among these three layers. The highest protein concentration was found in the aqueous C-serum, followed by rubber particles and lutoid particles in rank order (Yeang, Arif, Yusof, & Sunderasan, 2012).

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Proteins that are found in rubber latex function in regular energetic and defensive metabolic pathways of the plant cells. They also play crucial roles in *cis*-1,4-polyisoprene biosynthesis on the surface of the rubber particles (Rahman *et al*., 2013; Wititsuwannakul, Pasitkul, Kanokwiroon, & Wititsuwannakul, 2008; Yunyongwattanakorn, Tanaka, Sakdapipanich, & Wongsasuthiukul, 2008). There are several key enzymes involved with polymerization of the substrate, including isopentenyl pyrophosphate (IPP) to form long-chained polyisoprene. Activation of the substrates requires several key enzymes including 3- hydroxyl-3-methylglutaryl coenzyme A synthase (HMGS), 3- hydroxyl-3-methylglutaryl coenzyme A reductase (HMGR), and sucrose transporter (HbSUT) (Puskas, Gautriaud, Deffieux, & Kennedy, 2006; Suwanmanee, Sirinupong, & Suvachittanont, 2004; Tang *et al*., 2010). Other major enzymes involved in the polymerization process are *cis*-prenyl transferase (CIPT) and rubber elongation factor (Cornish & Xie, 2012; Puskas, Gautriaud, Deffieux, & Kennedy, 2006). However, due to complexity of the reactions, the complete mechanism of rubber biosynthesis remains to be elucidated. Currently, proteomic studies of rubber latex have been increasingly reported, in order to fill gaps in the understanding of the entire process of rubber biosynthesis (Dai *et al*., 2013; Gao *et al*., 2018; Habib *et al*., 2017; Wang *et al*., 2013; Xiang *et al*., 2012)

Various parameters are known to influence rubber latex production yield, including age of the tree, time for harvesting, geography, season, and treatments with plant hormones (Wang *et al*., 2015; Zhu & Zhang, 2009). It has been reported that ethylene treatment increases latex yield, dilution of the latex, and alteration of gene expression in aquaporins, sucrose transporters, hevein, glutamine synthetase, and HMGS (Broekaert, Lee, Kush, Chua, & Raikhel, 1990; Pujade-Renaud *et al*., 1994; Suwanmanee *et al*., 2004; Tungngoen *et al*., 2009; 2011; Zhu & Zhang, 2009). A recent report revealed that ethylene treatment of the rubber tree (clone RY 7-33-97) caused changes in the proteome and post-translational modifications (PTMs) of the rubber particle proteins. Upon stimulation, enzymes in carbohydrate metabolism, PTM activity, and hydrolase activity exhibited increased abundances; while proteins in the latex coagulation pathway showed decreased abundances (Wang *et al*., 2015). It was noted that the effectiveness of ethylene-releasing agents on boosting the rubber production, and their long-term deleterious effects, depend on the clonal variety of the tree (Lacote *et al*., 2010). Changes at the proteome level of crops by age of the tree have not been previously reported. Rubber plants can produce latex from about 6 years until about 32 years of age, although their lifetime can be longer than 100 years (Yogish, 2017). It has been reported that in the middle of their economic life, the rubber latex yield per tree is higher than that at an early or a late stage.

This work aimed to study the effects of ethylene-stimulating agent treatment and aging on alterations in the C-serum and rubber particle proteomes of *Hevea brasiliensis* (clone RRIM 600). The results from this report will contribute to understanding of the rubber biosynthesis processes at different ages, and following hormonal stimulation.

2. Materials and Methods

2.1 Harvesting of plant materials

Rubber latex was harvested from a plantation of *Hevea brasiliensis* trees of clonal variety RRIM600, in Rayong province, Thailand (GPS location: 13.013644, 101. 485642). Fresh latex was stored on ice during the one-hour transportation to the laboratory in Chonburi province, Thailand.

2.1.1 Treatment with 4-ethylphosphonic acid

Six rubber trees of the same age (13 years) and of equal stem girth were chosen for the experiments. Three plants were specified as control cases, and the other three were treated with an ethylene-stimulating agent 48 h prior to analysis. For the treated cases, 3.3% (v/v) 2-chloroethylphosphonic acid was applied directly on the scraped bark, under the tapping cut. In the untreated cases, water was applied to the scraped bark. After 48 h the rubber tapping was performed. The first twenty drops of rubber latex were discarded, and thereafter 20 mL of latex from each tree was collected in conical tubes and kept in the ice box.

2.1.2 Latex harvesting from plants of different ages

Three plantations of 8-year-old rubber trees, 15-year-old rubber trees, and 30-year-old rubber trees, all in the same area, were selected. Fresh latex samples were collected and pooled at 5.00 a.m. from each plantation, including approximately 100 trees per plantation. The latex samples were kept in conical tubes and stored on ice for an hour during transportation to the laboratories.

2.2 Determination of dry rubber content

Rubber latex was aggregated using absolute methanol in 1:10 (latex:methanol) ratio. The mixture was incubated at room temperature for 10 min and the clear supernatant was removed. Aggregated rubber was dried using vacuum centrifugation until constant weight was obtained. Dry rubber content is reported as the rubber weight per 1 mL latex solution.

2.3 Separation of C-serum proteins

Fresh latex (1 mL) was transferred to 1.5 mL tubes and centrifuged at 14,000 rpm for 30 min at 4°C. After this, the solution had separated into three layers. Rubber particles were aggregated on the top layer, followed by C-serum in the middle, and lutoid particles at the bottom. The C-serum was drawn from the middle using a 21G syringe needle. Protein concentration in C-serum was determined by Lowry's protein assay (Lowry, Rosebrough, Farr, & Randall, 1951).

2.4 Extraction of rubber particle proteins

Rubber particles were scraped from the top layer after centrifugation and incubated in 500 μL washing buffer

containing 10 mM Tris-HCl, 250 mM sucrose, at pH 7.0, for 30 min, followed by centrifugation at 14,000 rpm for 30 min at 4°C. The rubber particles were washed 3 times and subjected to extraction in a buffer containing 2% SDS, 62.5 mM Tris-HCl, at pH 6.8. The mixture was incubated at room temperature for 30 min with intermittent vortexing, followed by 30 s of sonication and 1 min of cooling on ice for 5 cycles. Samples were centrifuged at 14,000 rpm for 30 min at 4°C, and the supernatant was collected for protein concentration determination using the Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL).

2.5 Statistical analysis

In order to evaluate the effects of hormone treatment and plant age on dry rubber content and protein concentrations, statistical analysis was performed using Minitab software (version 17). One-way analysis of variance and Tukey's multiple comparison test were employed to compare the data between different plant ages, and a paired *t*-test was performed to assess effects of hormone treatment. The confidence level was set at 95% (p-value < 0.05) for calling statistical significance.

2.6 1D-gel electrophoresis and in-gel digestion

Forty micrograms of proteins from each sample and protein marker were subjected to SDS-PAGE using 12.5% separating polyacrylamide gels. Electrophoresis was performed at 100 V for 1.30 h. The protein bands were visualized using Coomassie blue staining. After that, in-gel digestion was performed following the protocol from Shevchenko, Tomas, Havlis, Olsen, and Mann (2006), with modifications. In brief, the gels were excised into 10 slices and dehydrated in 100% acetonitrile (ACN). Proteins in the gel pieces were reduced in 10 mM DTT and alkylated with 10 mM iodoacetamide. Digestion was performed with 100 ng trypsin in 10 mM NH_4HCO_3 at 37°C overnight. Digested peptides were extracted from the gel pieces using 50% ACN in 0.1% formic acid solution, followed by lyophilization to remove the extraction solution. The samples were kept at -20°C until use.

2.7 Liquid chromatography – mass spectrometry (LC-MS/MS)

Dried peptides were re-suspended in 0.1% formic acid and subjected to LC-MS/MS analysis. The samples were separated on a C18 PepSwiftTM monolithic nanocolumn (ThermoFisher Scientific, MA, USA) connected to an UltiMate 3000 HPLC system (Dionex Ltd., UK). The flow rate was set to be 300 nL/min. Eluent A was composed of 0.1% formic acid in H_2O, and eluent B was composed of 80% ACN and 0.1% formic acid in H_2O. Separation of peptides was performed for 20 min, including an equilibration step at 10% eluent A, a linear gradient from 10% to 70% eluent B for 13 min, and a regeneration step at 90% eluent B. Eluted peptides were detected with the HCT ion trap (Bruker Daltonics Ltd., Germany). Electrospray ionization source was used as an interface between the HPLC and mass spectrometer. Tandem mass spectrometry was performed in a data-dependent AutoMS (2) acquisition mode, which allows the five most abundant precursor ions to be selected for fragmentation by collision-induced dissociation (CID). Precursor ions were acquired at a scan range of 50-3000 *m/z*, and the range of product ions was set to be 300-1500 m/z with 3 scans averaged.

2.8 Data analysis

Acquired data were analyzed using Decyder MS 2.0 software (GE Healthcare, USA) connected to MASCOT server (Perkins, Pappin, Creasy, & Cottrell, 1999). For protein identification, the spectra were searched against the UniprotKnowledgeBase of Green plants (accessed May 2018). Threshold for protein identification was set as $p<0.05$. Protein quantification was performed using the differential analysis software embedded in Decyder MS 2.0. Proteins quantified with significant change in abundance are listed in this study, and were submitted to Gene Ontology Annotation using the Protein Information Resources (Wu *et al.*, 2003).

3. Results and Discussion

3.1 Effects of hormonal treatment on dry rubber and total protein contents

Ethephon or 2-chloroethylphosphonic acid is commonly used as an ethylene releaser in the natural rubber plantations to increase the amount of fresh latex per one harvest (Zhu & Zhang, 2009). After treatment with 2-chloroethylphosphonic acid for 48 h, the latex from ethylene treated plants showed slightly lower dry rubber content than the control (Figure 1(A)). The result is in agreement with previous reports that the ethylene stimulating agent causes dilution of the rubber latex and results in a larger amount of latex produced per tap (Tungngoen *et al*., 2011). Considering the total protein content, the amount of protein dissolved in C-serum did not change with elevated level of ethylene (Figure 1(B)); while the amount of protein embedded in the rubber particles increased upon ethylene treatment (Figure 1(C)). An increase in protein concentration in the rubber particles could be possibly caused by ethylene stimulation inducing a larger population of comparatively small rubber particles (Wang *et al*., 2015). This would increase the specific surface area, and when the proteins on surfaces are readily extracted by the 2% SDS solution result in an apparently higher protein concentration.

3.2 Effects of plant maturation on dry rubber and total protein contents

Maturation of the rubber plant affects the amount of latex produced. It has been reported that fresh latex production by rubber trees at the age of 16 yrs is approximately twice that at 12 yrs or at 26 yrs (Chiarawipa & Prommee, 2013). Our results indicate that 30 yrs old plants generated latex with a higher dry rubber content than the 15 or 8 yrs old plants (Figure 1(D)). Around the midlife at 15 yrs of age, where the highest latex yield is observed, the soluble protein concentration in the C-serum was found to be the lowest among the tested age groups (Figure 1(E)); whereas the concentration of membrane - bound proteins on the rubber

particles was found to be the highest (Figure 1(F)). It is hypothesized that the rubber particles at the age of 15 yrs have a larger fraction of active rubber particles; however, more experimentation is needed to prove this hypothesis.

3.3 Ethylene treatment affects protein profiles and abundances

Protein profiles from C-serum and rubber particle proteins were affected by ethylene stimulation as shown in coomassie blue-stained SDS-PAGE gels (Figure 2(A) and Figure 2(B)). Obvious changes in abundance were observed in the proteins extracted from the rubber particles; while slight changes were shown in the SDS-PAGE profile of the C-serum proteins. To identify the types and abundances of proteins from the gels, bottom-up proteomic experiments were performed. The gels were excised and subjected to in-gel tryptic digestion prior to LC-MS/MS analyses using the HCT ion trap. After comparing the MS/MS spectra for matches against the green plant database and comparing signal intensity of the precursor ions using Decyder 2.0 software, the results indicated that ethylene stimulation altered abundances of 1,553 C-serum proteins, of which 768 proteins had increased abundance and 785 proteins had decreased abundance. Of those affected proteins, 1,507 were common to the two samples, whereas 16 unique proteins were identified solely in untreated samples, and 30 proteins were uniquely expressed with the treatment of ethylene (Figure 3(A)). Regarding the proteins extracted from rubber particles, 1,001 were quantified as changed in abundance upon treatment with the ethylene stimulating agent, with 469 having higher and 532 having lower abundance. Considering the types of proteins identified, 10 proteins were unique to the control sample, whereas 6 proteins were solely present in the treated sample (Figure 3B). It was obvious that most of the altered proteins were common, identified in both the untreated and the treated samples. However, most altered proteins were not shared between C-serum and rubber particles (Figure 3(C)), confirming the necessity of separating these components of the rubber latex. A group of altered proteins associated with the keyword "ethylene" is indicated in Table 1 and Table 2, showing the more and the less abundant proteins, respectively.

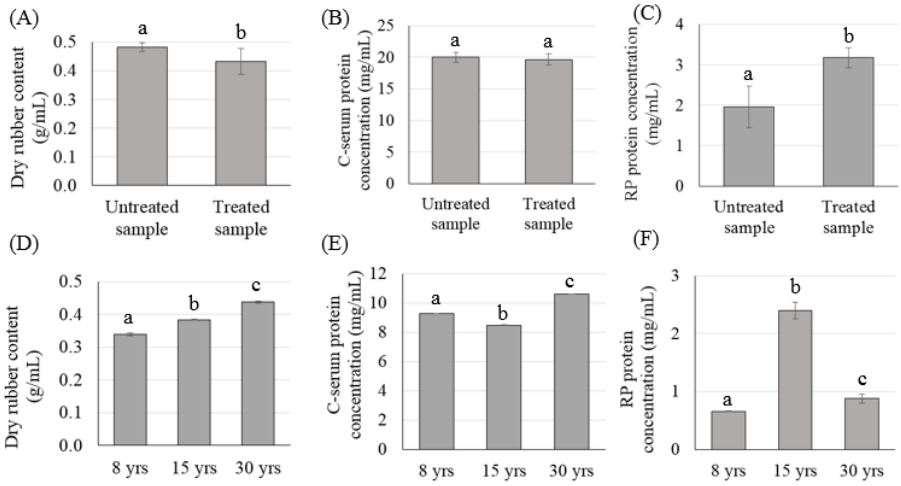

Figure 1. Effects of 2-chloroethylphosphonic acid treatment on (A) dry rubber content, (B) protein concentrations in C-serum, and (C) protein concentration in rubber particles. And, effects of plant ages on (D) dry rubber content, (E) protein concentrations in C-serum, and (F) protein concentration in rubber particles. Data are shown as mean ± standard deviation from at least triplicate experiments. Different letters (a-c) indicate significant differences at $p < 0.05$.

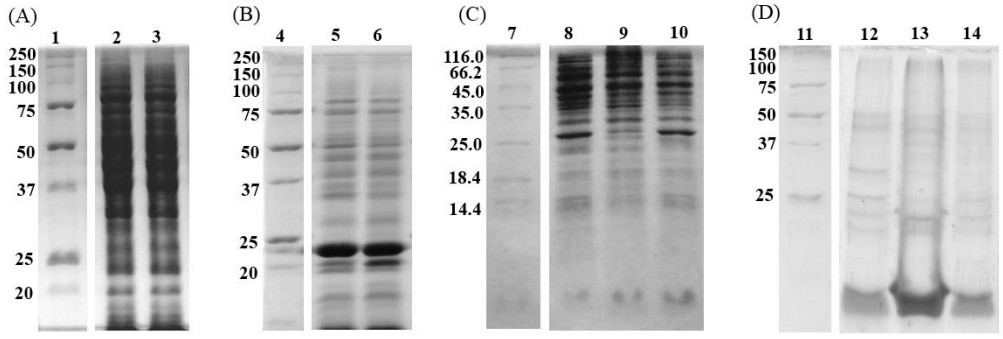

Figure 2. SDS-PAGE showing changes in protein profiles from different conditions. (A) C-serum proteins altered by 2- chloroethylphosphonic acid treatment, (B) rubber particle proteins altered by 2- chloroethylphosphonic acid treatment, and (C) C-serum proteins from different ages of plants. (D) rubber particle proteins from different ages of plants. Lanes 1, 4, 7, 11: molecular weight markers, lanes 2, 5: 2- chloroethylphosphonic acid - untreated sample, lanes 3, 6: 2-chloroethylphosphonic acid treated samples, lanes 8, 12: samples from 8-year-old plants, lanes 9, 13: samples from 15-year-old plants, and lanes 10, 14: samples from 30 years old plants.

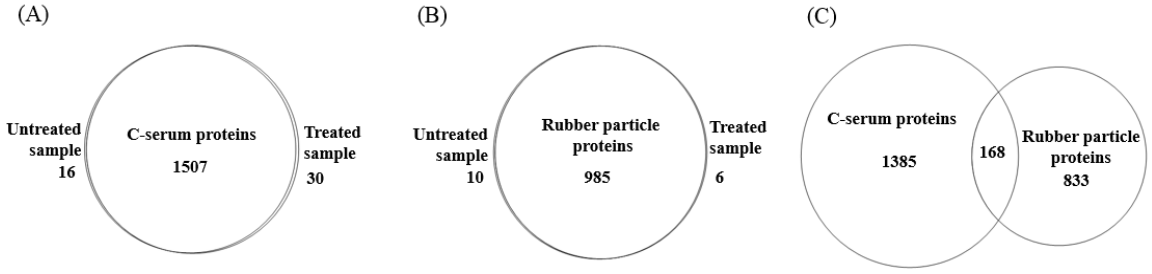

Figure 3. Comparison of numbers of proteins identified from (A) untreated and 4-chloroethylphosphonic acid treated C-serum proteins, (B) untreated and 4-chloroethylphosphonic acid treated rubber particle proteins, and (C) total proteins from C-serum and rubber particles.

Table 1. List of ethylene-related proteins that had increased spectral intensities in the ethylene stimulated samples.

No.	Protein	Accession number	Reference database	Log_2 (intensity)		Location
				Untreated sample	Treated sample	
1	Ethylene-responsive transcription factor 1A	O80337	Arabidopsis thaliana	6.83	12.17	C-serum
2	Ethylene receptor 4	Q6T5K3	Oryza sativa subsp. indica	8.11	10.87	C-serum
3	Ethylene response sensor 2	P93825	Arabidopsis thaliana	8.98	10.25	C-serum
4	Ethylene-responsive transcription factor ERF061	Q9C7W2	Arabidopsis thaliana	7.78	9.01	C-serum
5	Ethylene-responsive transcription factor ERF035	Q9M210	Arabidopsis thaliana	9.51	10.31	C-serum
6	Ethylene-induced calmodulin-binding protein b	Q9FY74	Arabidopsis thaliana	4.86	10.31	Rubber particle
7	Ethylene-forming enzyme	Q43792	Nicotiana tabacum	8.59	13.15	Rubber particle
8	Ethylene-responsive transcription factor ERF120	Q9SK67	Arabidopsis thaliana	11.18	11.53	Rubber particle

Table 2. List of ethylene-related proteins that had decreased spectral intensities in the ethylene stimulated samples.

No.	Protein	Accession number	Reference database	Log_2 (intensity)		Location
				Untreated sample	Treated sample	
1	Ethylene-forming enzyme	A2Z1W9	Oryza sativa subsp. indica	8.84	8.44	C-serum
2	Ethylene-responsive transcription factor ERF034	Q8LBQ7	Arabidopsis thaliana	10.56	10.10	C-serum
3	Ethylene receptor 1	Q9SSY6	Cucumis sativus	10.19	9.91	C-serum
4	Ethylene-responsive transcription factor CRF2	Q9SUQ2	Arabidopsis thaliana	10.28	9.62	C-serum
5	Protein ethylene- dependent gravitropism-deficient and yollow-green	Q949Y5	Arabidopsis thaliana	10.35	9.39	C-serum
6	AP2-like ethylene-responsive transcription factor SNZ	Q6PV67	Arabidopsis thaliana	10.86	9.49	C-serum
7	AP2-like ethylene-responsive transcription factor TOE2	Q9LVG2	Arabidopsis thaliana	12.02	6.60	C-serum
8	Ethylene-responsive transcription factor ERF119	Q9LUA2	Arabidopsis thaliana	8.37	6.98	Rubber particle
9	AP2-like ethylene-responsive transcription factor SNZ	Q6PV67	Arabidopsis thaliana	9.42	7.91	Rubber particle
10	Ethylene receptor 4	Q6T5K3	Oryza sativa subsp. indica	13.07	11.59	Rubber particle

The green plant database used for data interpretation shows that these proteins have been earlier identified in other species, not including *Hevea brasiliensis*. It is possible that many of the ethylene responsive proteins have not been previously identified in *Hevea brasiliensis*, so the use of a larger database is crucial. It is interesting that the two key

proteins involved with polyisoprene elongation were affected by ethylene. The abundances of rubber elongation factor (REF) and small rubber particle protein (SRPP) increased in the C-serum with the presence of ethylene; while the abundances of these two proteins in the rubber particles decreased.

3.4 Gene ontology annotation of proteins with altered abundances in response to ethylene

Following identification and comparison of abundances of proteins found in C-serum and in rubber particles, between the 4-chloroethylphosphonic acid treated and the untreated rubber plants, gene ontology annotation (GOA) was performed to classify "molecular function", "biological process" and "cellular component" of the differentially expressed proteins. The analysis was performed using the group of proteins quantified with the highest abundance from each tested age group of rubber trees. The results indicate that the majority of identified proteins from both C-serum and rubber particles participated in binding activities (Figure 4(A) and Figure 4(B)) and played a role in response to stimulus (Figure 4(C) and Figure 4(D)). The most frequent subcellular location of proteins in both types of samples was shown to be membrane (Figure 4(E) and Figure 4(F)). It was found that the frequencies of proteins assigned to each GO-term differed between c-serum and rubber particles. For example, a larger proportion of proteins with the term "ion-binding" was observed among those of higher abundance in C-serum, than in the lower abundance proteins (Figure 4(A)); while the opposite pattern was observed for proteins identified from rubber particles.

3.5 Plant maturation affects protein profiles and abundances

Effects of the age of rubber plants on protein expression profiles were demonstrated using SDS-PAGE (Figure 2(C)). There were several coomassie blue-stained bands with intensities differing by age of the plant. It was noticeable that C-serum protein profiles shown in Figure 2(C) were different from Figure 2(A); and rubber particle protein patterns shown in Figure 2(D) and Figure 2(B) are also dissimilar. This observation emphasizes the fact that many parameters, including latex harvesting season and age of the plants, could affect the protein profiles in latex. Therefore, the changes in protein abundance should be compared only within a controlled set of experiments. The gel bands were excised and subjected to in-gel digestion and tandem mass spectrometry analysis. Based on MS/MS ion search against the green plant database followed by comparative quantitative analysis, it was found that 2,961 proteins exhibited changes in abundance level in the cytoplasm of laticifer cells. The majority of these were identified in all three types of samples (2,449 proteins) as shown in Figure 5(A). There were 506, 293 and 1088 proteins that showed their highest abundance in 8 yrs, 15 yrs and 30 yrs old plants, respectively. Several proteins involved with rubber biosynthesis were found to be suppressed at the mature age of 30 yrs, including the rubber elongation factor (REF) and the small rubber particle protein (SRPP), which play a major role in the polyisoprene elongation process. In addition, pro-hevein was found to be up-regulated at the mature age of 30 yrs. It is the precursor of hevein, which is known to be the major cause of latex coagulation (Gidrol, Chrestin, Tan, & Kush, 1994). Aggregation of the rubber particles could be a reason why dry rubber content in latex from 30 yrs-old plants was found to be the highest among the three sampled ages of the trees.

Regarding the proteins embedded in the rubber particles, 839 of these were identified with changes in protein abundance level. In the Venn diagram in Figure 5(B), most proteins are seen to overlap all three samples (750 proteins). Of all the listed proteins, the abundances of 163, 574, and 102 proteins were found to be the highest at the ages of 8 rys, 15 yrs, and 30 yrs, respectively. Similar to the soluble C-serum proteins, the abundance of SRPP embedded in the rubber particles from the 30-yrs old trees was shown to be the lowest among the tree age groups. However, no change in abundance of pro-hevein was observed on the rubber particles. Considering the groups of altered proteins identified from C-serum and rubber particle extracts, only 136 proteins were shared, while most of the proteins were uniquely identified in each part of the latex (Figure 5(C)).

3.6 Gene ontology annotation of proteins with altered abundances in response to plant maturation

Functional analysis of affected proteins quantified with the highest abundances at plant ages of 8 yrs, 15 yrs, and 30 yrs were characterized by GOA, as shown if Figure 6. Most of the proteins with changes in abundance from both C-

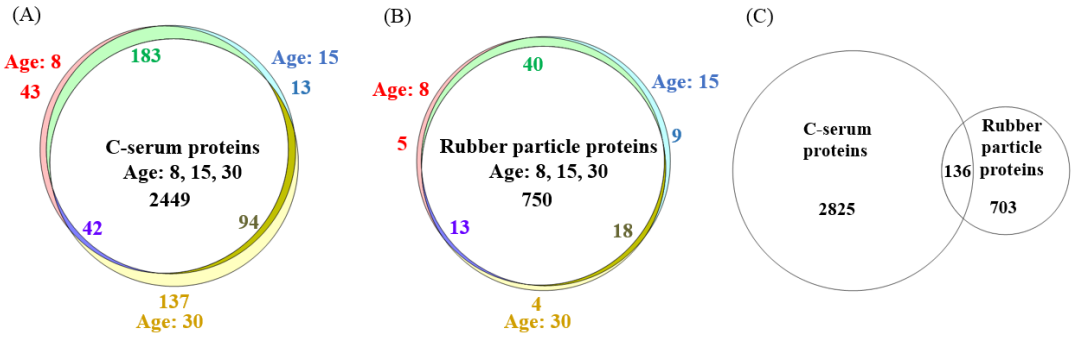

Figure 5. Comparison of numbers of proteins identified from (A) C-serum proteins from 8 yrs, 15 yrs, and 30 yrs old trees' latex, respectively, (B) rubber particle proteins from 8 yrs, 15 yrs, and 30 yrs old trees' latex, respectively, and (C) total proteins from C-serum and rubber particles.

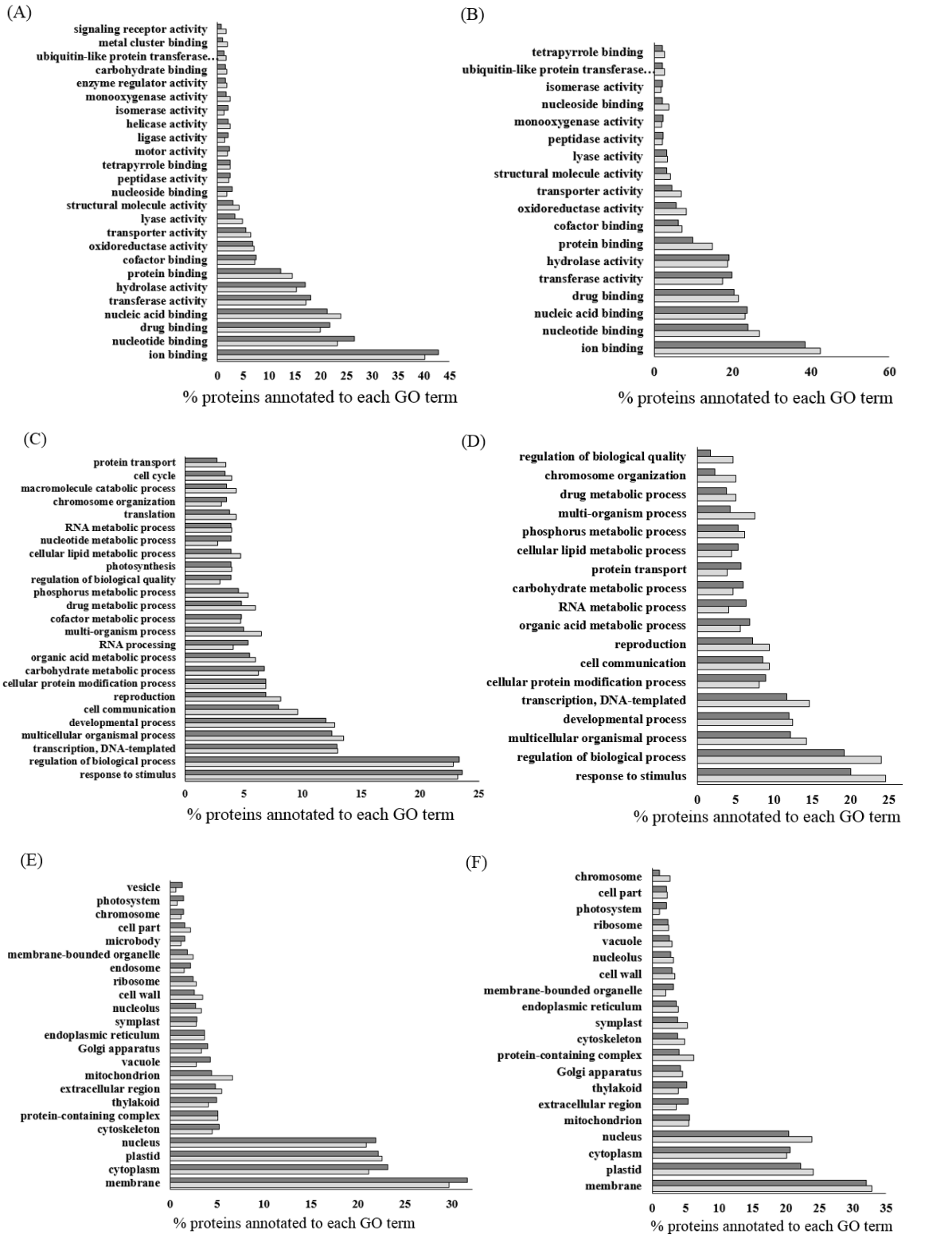

Figure 4. Gene Ontology Annotation indicating (A) molecular functions of C-serum proteins, (B) molecular functions of rubber particle proteins, (C) biological processes of C-serum proteins, (D) biological processes of rubber particle proteins, (E) cellular locations of C-serum proteins, and (F) cellular locations of rubber particle proteins. The symbol ■ represents proteins with higher abundance in response to 2-chloroethylphosphonic acid and □ represents proteins with lower abundance in response to 2-chloroethylphosphonic acid.

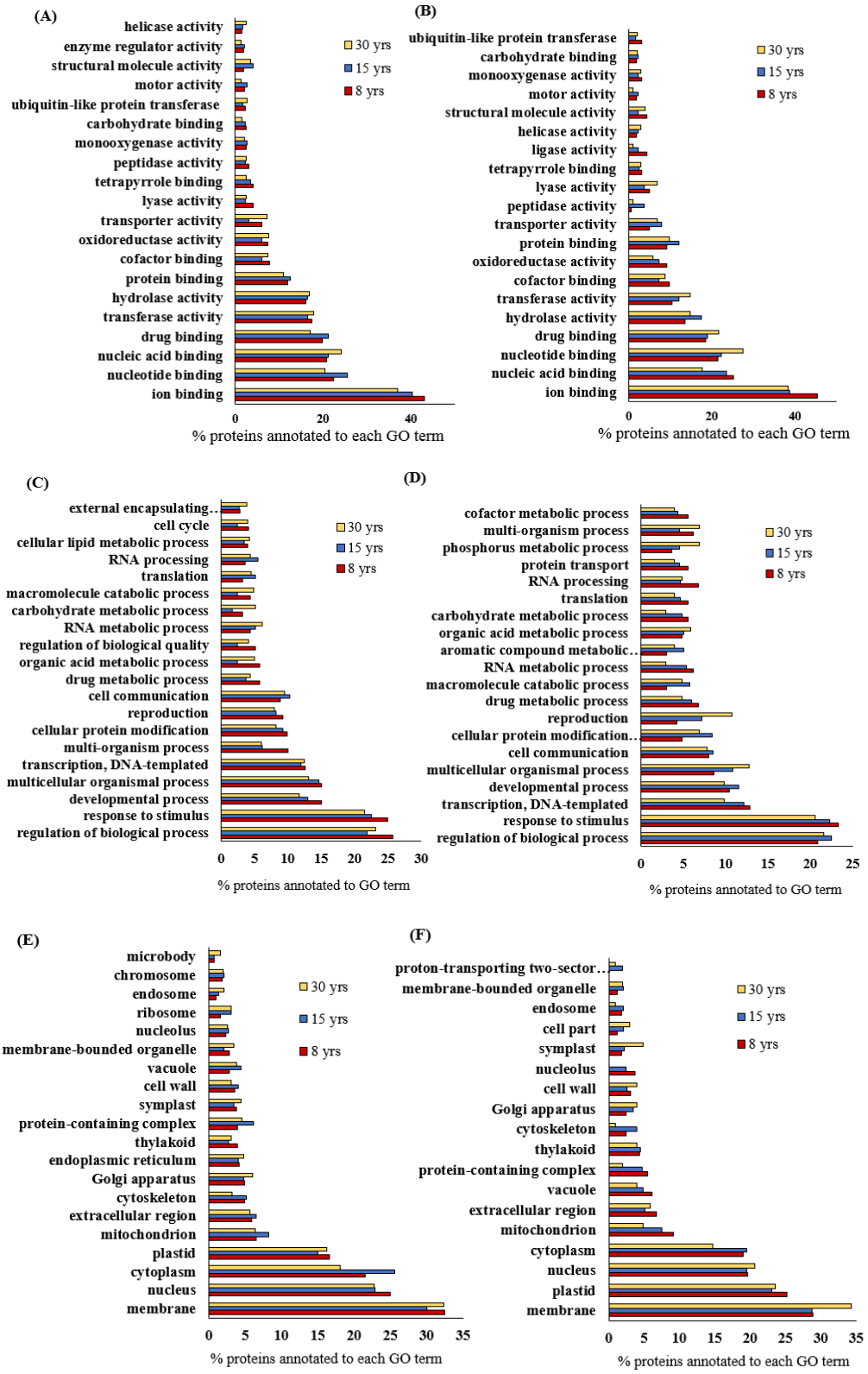

Figure 6. Gene Ontology Annotation indicating (A) molecular functions of C-serum proteins, (B) molecular functions of rubber particle proteins, (C) biological processes of C-serum proteins, (D) biological processes of rubber particle proteins, (E) cellular locations of C-serum proteins, and (F) cellular locations of rubber particle proteins from different age groups of rubber trees.

serum and rubber particle components showed molecular functions associated with the term "ion binding". It is obvious that the younger rubber plants expressed more "ion binding" proteins than the older ones (Figure 6(A) and Figure 6(B)). As regards biological process, the two terms with the largest numbers of proteins identified in C-serum and in rubber particle samples were "regulation of biological process" and "response to stimulus", respectively. Comparing between the age groups, the younger plants expressed a larger proportion of proteins involved with "regulation of biological process" and "response to stimulus" than the older plants (Figure 6(C) and Figure 6(D)). Regarding cellular location, the majority of affected proteins from both C-serum and rubber particles were located on "membrane". The largest proportion of "membrane" proteins were identified from the samples with the highest abundance at the age of 30 yrs (34.3%) (Figure 6(E) and Figure 6(F)). Many of these membrane proteins had binding activities, which is in agreement with the molecular functions chart in Figure 6(B).

4. Conclusions

Our results suggest that treatment with 4-chloroethylphosphonic acid and maturation of the rubber plant both affect the dry rubber content, and the concentrations of proteins, especially of those proteins embedded in the rubber particles. In proteomic analysis, most of the identified proteins with affected abundances were uniquely found in either C-serum or rubber particle fractions of the latex. Many of the ethylene responsive proteins have not been previously reported, and could be targets of interest for further studies. Our results indicate that the maturation of rubber trees reduces the abundance of polyisoprene elongation -related proteins, and increases the abundance of coagulation-related proteins. This study provided proteomics information about how the rubber trees respond to ethylene treatment and aging, which are related to rubber latex yields on plantations.

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