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Full Length Research Paper

Proteome analysis of interaction between rootstocks and scions in *Hevea brasiliensis*

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The main propagation method of rubber tree (*Hevea brasiliensis* Muell. Arg.) is by grafting. However, the molecular mechanism underlying rootstock-scion interactions remains poorly understood. Identification and analysis of proteins related to rootstock-scion interactions are the bases of clarifying the molecular mechanism underlying rootstock-scion interactions. To identify proteins associated with rootstock-scion interactions, *in vitro* plantlets from immature anthers of *Hevea brasiliensis* Reyan 88-13 (R.88-13) and Haiken 2 (H.2) were used to produce two scion/rootstock combinations, namely R.88-13/H.2 and H.2/R.88-13. In this study, the bark proteins of R.88-13/H.2 scions, H.2/R.88-13 rootstocks and the ungrafted R.88-13 (control) were extracted and separated by two-dimensional gel electrophoresis (2-DE), respectively. 48 differentially expressed protein spots were obtained and subjected to matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) followed by database searching. 38 protein spots were successfully identified and these proteins are mainly involved in response to stimulus, metabolism and photosynthesis. Some of the identified proteins may be closely correlated with rootstock-scion interactions, whose possible functions in rootstock-scion interactions were discussed.

Key words: Rubber tree, MALDI-TOF MS, rootstock-scion interaction, proteome.

INTRODUCTION

Rubber tree (*Hevea brasiliensis* Muell. Arg.) is a very important perennial crop which produces natural rubber. At present, the main propagation method of rubber tree is by grafting, while the seeds are mostly used for the production of rootstocks. Many experiments have demonstrated that there are interactions between rootstocks and scions of rubber tree in growth and rubber yield (Ahmad, 1999; Cardinal et al., 2007), physiology and biochemistry (Sobhana et al., 2001) and endogenous phytohormones (Lin et al., 2005). However, the study about the molecular mechanism underlying rootstock-scion interactions in rubber tree has not been reported. In other plants, such as the apple and tomato, the effects of rootstocks on gene expression patterns of scions have been studied (Jensen et al., 2003, 2010; Zhang et al., 2008). Using cDNA-amplified fragment length polymorphism technique, Jensen et al. (2003) found that Gala scions grafted to the M.7 EMLA rootstocks showed increased stress-related gene expression compared to Gala scions grafted to the M.9 T337 rootstocks, which is correlated with physiological differences between M.7 EMLA (moderately resistant to fire blight) and M.9 T337 (highly susceptible to fire blight). Furthermore, Jensen et al. (2010) evaluated the influences of seven different rootstocks that produced a range of tree sizes, from extremely dwarfed to highly vigorous, on gene expression of Gala scions by the DNA microarray. Results showed that different rootstocks triggered distinct, reproducible scion gene expression patterns and 2934 scion transcripts had differential expression, of which, 116 transcripts were associated with tree size. In addition, the tomato rootstocks regulated gene expression of the eggplant scions. The regulated genes were involved in many

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cellular functions, such as metabolism, signal transduction and stress-response (Zhang et al., 2008). All the above results provide a good theoretical reference for studying the rootstock-scion interactions of rubber tree from a molecular perspective.

Elucidating the molecular mechanism underlying rootstock-scion interactions is helpful to reasonably selecting of rootstocks and scions and cultivating good rubber tree clones. The objective of this study was to identify the proteins correlated with rootstock-scion interactions. This will provide a theory base for further clarifying the molecular mechanism underlying rootstock-scion interactions of rubber tree.

MATERIALS AND METHODS

Plant materials

In vitro plantlets from immature anthers of *Hevea brasiliensis* R.88-13 (RRIM600×PilB84) and H.2 (PB86×PR107) were transplanted in the Experimental Field of the Chinese Academy of Tropical Agriculture Science in 1997. Next year R.88-13 and H.2 were inter-grafted, producing two scion/rootstock combinations, namely R.88-13/H.2 and H.2/R.88-13. In this experiment, the ungrafted plants of R.88-13 were used as the control. All the plants were maintained under the same production managements in the whole growing period, namely weeding one time a year and all the plants not tapping. Five plants from every grafting combination and the control were selected as the experiment materials. The barks of R.88-13/H.2 scions, H.2/R.88-13 rootstocks and the control were collected in the same time in 2008, frozen in liquid nitrogen and stored in −80°C until protein extraction.

Protein extraction

The barks of R.88-13/H.2 scions, H.2/R.88-13 rootstocks and the control were pooled, respectively and ground into fine powder with liquid nitrogen. The powder was then transferred to a centrifuge tube containing extraction buffer (5 mol/L urea, 2 mol/L thiourea, 2% w/v 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), 40 mmol/L Tris) and incubated at 4°C for 4 h. Centrifugation was performed at 18 000×g for 30 min at 4.0°C to pellet all insoluble materials, which was subsequently discarded. The supernatant was transferred to a fresh tube and mixed with 10 volumes of chilled 10% w/v trichloroacetic acid (TCA)/acetone solution and proteins were precipitated at –20.0°C for 4.00 h. After centrifugation at 18 000×g for 30 min at 4.0°C, the supernatant was discarded and the pellet was rinsed with chilled acetone, followed by incubation at –20.0°C for 2.00 h. Precipitated protein was pelleted by centrifugation at 18000×g for 30 min at 4.0°C. The cleaning process was repeated twice. The final pellets were dried at room temperature and resuspended in lysis buffer (9 mol/L urea, 4% w/v CHAPS, 1% v/v pH 3–10 ampholytes (Bio-Rad), 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 10 mmol/L dithiothreitol (DTT)). Protein concentration was determined by the Bradford assay (BioRad, Hercules, CA, USA) with bovine serum albumin (BSA) as the standard. Samples were stored at –80.0°C until isoelectric focusing (IEF).

2-DE and image analysis

Immobilized pH gradient (IPG) strips (17 cm, 4–7 linear pH gradient; Bio-Rad) were rehydrated for 12 h in 300 µL rehydration buffer (8 mol/L w/v urea, 2% w/v CHAPS, 40 mmol/L DTT, 0.6% v/v pH 3–10 ampholytes, 0.002% w/v bromophenol blue). 200 µg of protein were loaded on each lane. Three gel replicates were performed for each sample. IEF was carried out on a Protean IEF Cell (Bio-Rad) at 20.0°C with current limit 50 µA/strip until 60 000 Vh. After Isoelectric focusing (IEF), IPG strips were stored at –20.0°C or immediately equilibrated as described by Görg et al. (1987). The second dimension sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 12% polyacrylamide gels using Protean II XI Cell (Bio-Rad). The electrophoresis run at 15 mA for 30 min plus 30 mA until the dye front reached the bottom of the gels. Proteins were visualized by silver staining (Blum et al., 1987) and images were digitalized with a GS-800 Calibrated Densitometer (Bio-Rad). Image analysis was performed using PDQuest^{TM} software (Bio-Rad). Spot matching was checked and corrected manually. The intensity of each protein spot was normalized relative to the total abundance of all valid spots. Protein expression levels were compared among the R.88-13/H.2 scions, H.2/R.88-13 rootstocks and the control. Protein spots with at least three-fold increase/decrease were considered as differentially expressed ones.

Protein digestion and identification by MALDI-TOF MS

Differentially expressed protein spots were manually excised from silver stained gels using pipette tips, digested with trypsin and measured using an UltraflexII TOF/TOF MALDI Mass Spectrometer (Bruker-Daltonics) as described earlier (Yuan et al., 2008). The Flexanalysis software (Bruker-Daltonics) was used to analyze peptide mass fingerprints (PMFs) spectra which were calibrated using trypsin autolysis peaks (m/z 842.51 and m/z 2211.10) as internal standards. The NCBInr database was searched for the acquired PMF data using the Mascot software available at (http://www.matrixscience.com). The searching parameters were set according to Yuan et al. (2008) except that Gln->pyro-Glu (N-term Q) and oxidation (M) were used as variable modifications. For a positive protein identification, at least five peptides were matched and the score was more than 70 (p<0.05). Priority was given to the identified proteins from woody plant species.

RESULTS

Differential expression of proteins

Proteins were extracted from barks and separated using 2-DE followed by silver staining. The protein patterns were analyzed by PDQuest^{TM} software (Bio-Rad). Each experiment was repeated three times. The analyses led to the detection of 268±11 at control (0), 338±44 at H.2/R.88-13 rootstocks (1) and 319±23 spots at R.88-13/H.2 scions (2) (Figure 1). The pair wise comparisons were marked with 0/1, 0/2 and 1/2 (0 and 1 used as the reference gels, respectively). A total of 48 spots displayed differential expression in intensities (P<0.05), including 22 spots for 0/1 comparison, 15 for 0/2 and 19 for 1/2, among which, 8 spots showed differential expression in two of three groups of comparisons, namely spots 41, 1-63, 1-68, 144, 129, 145, 231 and 21 (Figure 1).

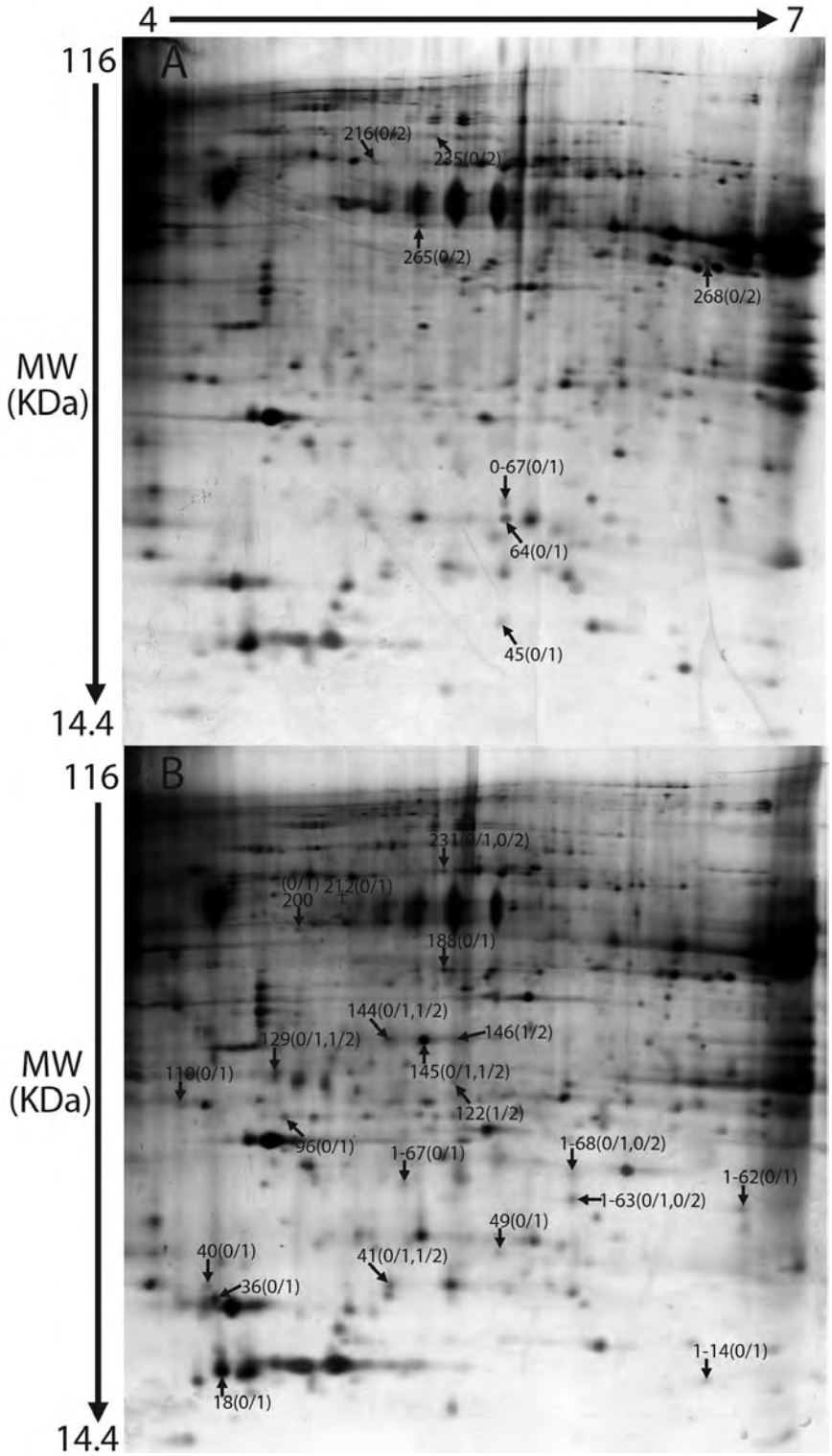

Figure 1. Comparison of the protein patterns of rubber tree barks. 200 µg of protein were loaded on a pH 4–7 linear 17 cm IPG strip followed by a 12% SDS-PAGE. The protein spots were visualized with silver staining. A, B and C displayed bark protein patterns of control (0), H.2/R.88-13 rootstock (1) and R.88-13/H.2 scion (2), respectively. Pairwise comparisons were performed among 0, 1 and 2, namely 0/1, 0/2 and 1/2 (0 and 1 used as the reference gels, respectively). The numbers were corresponding to Table 1 and arrows indicated differentially expressed protein spots which were subjected to MALDI-TOF MS followed by database searching.

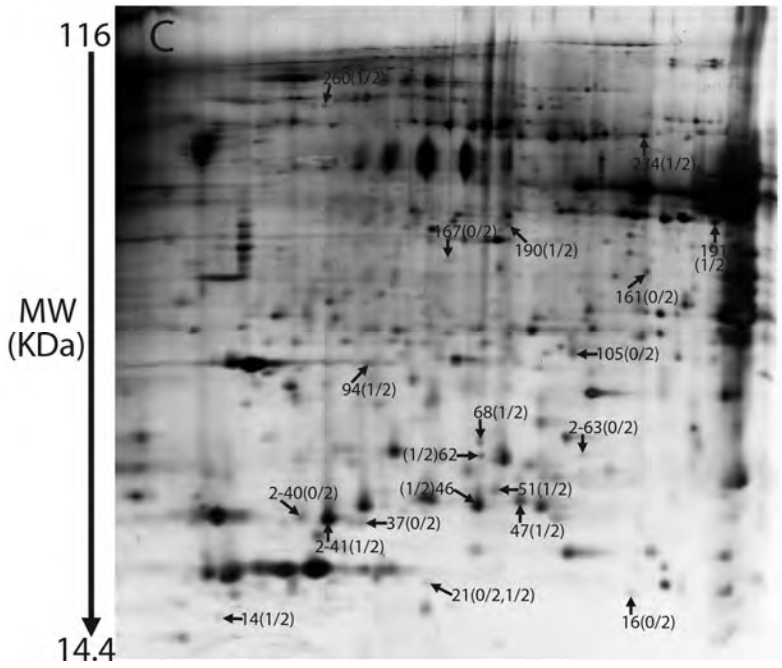

Figure 1. Contd.

MALDI-TOF MS and protein identification

All the 48 differentially displayed protein spots were subjected to MALDI-TOF MS followed by database searching, of which 38 acquired positive identifications except for spots 14, 18, 21, 45, 51, 122, 129, 146, 161 and 212, listed in Table 1.

Compared with the ungrafted R.88-13, protein spots that newly appeared or up-regulated in the H.2/R.88-13 rootstocks (0/1) were identified as heat shock factor (1-63), Os11g0597700 (1-67), VILIiN related family member (40), photosystem II protein 33kD (144), predicted proteins (1-68 and 96), hypothetical proteins (36 and 41), and oxygen-evolving enhancer protein 1(145). Spots with disappearance or down-regulation were corresponding to HEV2.1 (0-67 and 64), putative mudrA protein (1-62), putative retroelement pol polyprotein (49), mitochondrial F1-ATPase beta subunit (231) and other hypothetical and predicted proteins (1-14, 110, 188 and 200).

Compared with the ungrafted R.88-13, protein spots that newly appeared or up-regulated in the R.88-13/ H.2 scions (0/2) were 1-63, 1-68, 2-40, 37, 105 and 16. Of them, spots 37 and 105 were matched to theta class glutathione transferase GSTT1 and taxadiene synthase, respectively, while 2-40 and 16 were both hypothetical proteins. Spots with disappearance or down-regulation were 216, 235, 265, 268, 231, 2-63 and 167. Spot 216 and 265 were identified as the mixture of two proteins. The former corresponded to class III peroxidase and maturase K, and the latter to hypothetical protein OsI_31756 and hypothetical protein SORBIDRAFT_ 05g005061. Other spots were predicted protein (235 and 2-63), unknown (268) and unnamed protein product (167).

Compared with the H.2/R.88-13 rootstocks, protein spots that newly appeared in the R.88-13/H.2 scions (1/2) had similarities to putative At14a-2 protein (62), HEV2.1 (68), small rubber particle protein (94), peroxidase (190) and phosphoenolpyruvate carboxylase (260). Spots with up-regulation were matched to unnamed protein product (2-41), Os06g0136700 (46), calcineurin B-like protein 9 (47), phytochrome B (191) and hypothetical protein (41 and 234). Spots 144 and 145 showed disappearance and down-regulation, corresponding to photosystem II protein 33kD and oxygen-evolving enhancer protein 1, respectively. The detailed information of identified protein spots are presented in Table 1.

Functional categories of differentially expressed proteins

For 38 differentially expressed proteins with positive identifications, possible functions can be assigned based on their similarities with previously characterized proteins, which were mainly from other species. The proteins were classified into five main categories, including unknown (52.5%), response to stimulus (17.5%), metabolism (15%), photosynthesis (7.5%) and other functional proteins (7.5%, Figure 2).

DISCUSSION

In this experiment, *in vitro* plantlets from R.88-13 and H.2

Table 1. Identification of differentially expressed proteins by PMFs.

Spot number	Protein description	Accession number	MW[a)] (kDa)/pI [a)]	Score	Matched peptides	Sequence coverage (%)	Species	Possible function	Variation		
									0/1	0/2	1/2
1-63	Heat shock factor	gi|229473708	44/5.43	72	7/20	12	*Boea hygrometrica*	response to stimulus	+	+	
1-67	Os11g0597700	gi|255680240	50/9.08	75	8/39	21	*Oryza sativa Japonica Group*	unknown	+		
1-68	Predicted protein	gi|168001070	66/9.91	85	11/39	20	*Physcomitrella patens* subsp. *patens*	unknown	+	+	
36	Hypothetical protein CHLREDRAFT_169274	gi|159464233	572/5.61	71	39/81	8	*Chlamydomonas reinhardtii*	unknown	+		
40	VILIiN related family member (viln-1)	gi|17505552	105/7.22	83	11/32	13	*Caenorhabditis elegans*	organelle organization	+		
41	hypothetical protein	gi|147778396	130/9.11	107	24/74	19	*Vitis vinifera*	unknown	+		↑
96	Predicted protein	gi|226460827	25/7.96	77	7/35	31	*Micromonas pusilla CCMP1545*	unknown	+		
144	Photosystem II protein 33kD	gi|224916	27/5.01	70	7/37	29	*Spinacia oleracea*	photosynthesis	+		−
145	Oxygen-evolving enhancer protein 1, chloroplastic	gi|131386	35/5.58	111	12/87	36	*Spinacia oleracea*	photosynthesis	↑		↓
0-67	HEV2.1	gi|37954950	23/5.63	78	7/27	32	*Hevea brasiliensis*	response to stimulus	−		
64	HEV2.1	gi|37954950	23/ 5.63	86	8/67	32	*Hevea brasiliensis*	response to stimulus	−		
1-62	Putative mudrA protein - maize transposon MuDR	gi|14488305	109/8.83	72	13/60	14	*Oryza sativa*	metabolism	↓		

Table 1. Continue.

1-14	Hypothetical protein SORBIDRAFT_02g026980	gi|242049526	98/7.26	72	16/81	17	*Sorghum bicolor*	unknown	↓		
49	Putative retroelement pol polyprotein	gi|4567277	154/8.01	80	18/56	15	*Arabidopsis thaliana*	DNA integration	↓		
110	Hypothetical protein POPTRDRAFT_835735	gi|224141559	93/6.23	72	14/52	16	*Populus trichocarpa*	unknown	↓		
188	hypothetical protein	gi|147800264	138/8.76	70	14/33	11	*Vitis vinifera*	unknown	↓		
200	Predicted protein	gi|224124120	81/ 4.75	80	16/59	20	*Populus trichocarpa*	unknown	↓		
231	Mitochondrial F1-ATPase beta subunit	gi|269914683	60/6.18	98	16/59	27	*Dimocarpus longan*	metabolism	↓	↓	
2-40	Hypothetical protein	gi|147769682	29/ 9.76	77	8/30	42	*Vitis vinifera*	unknown		+	
37	Theta class glutathione transferase GSTT1	gi|283135892	28/9.37	71	9/49	28	*Populus trichocarpa*	metabolism		+	
105	Taxadiene synthase	gi|83596265	99/5.35	72	15/60	21	*Taxus cuspidata*	metabolism		+	
16	Hypothetical protein	gi|147792147	87/9.36	71	8/22	12	*Vitis vinifera*	unknown		↑	
216	Class III peroxidase	gi|211906542	37/9.34	140	7/44	21	*Gossypium hirsutum*	response to stimulus		–	
	Maturase K	gi|90193149	53/9.75		5/44	10	*Disa chrysostachya*	metabolism			
	Hypothetical protein OsI_31756	gi|218202326	155/8.41		21/67	16	*Oryza sativa Indica Group*	unknown			
265	Hypothetical protein SORBIDRAFT_05g005061	gi|242070283	88/ 8.78	130	13/67	20	*Sorghum bicolor*	unknown		–	
235	Predicted protein	gi|168044454	74/ 8.66	74	12/35	15	*Physcomitrella patens* subsp. *patens*	unknown		–	
268	Unknown	gi|21536713	43/5.87	72	8/33	20	*Arabidopsis thaliana*	unknown		–	
2-63	predicted protein	gi|168046266	141 /8.77	81	21/67	14	*Physcomitrella patens* subsp. *patens*	unknown		↓	
167	unnamed protein product	gi|9759529	133/ 5.40	80	17/53	14	*Arabidopsis thaliana*	unknown		↓	
62	Putative At14a-2 protein	gi|26451448	24/5.50	86	9/80	31	*Arabidopsis thaliana*	unknown			+
68	HEV2.1; hevein	gi|37954950	23/5.63	78	7/69	32	*Hevea brasiliensis*	response to stimulus			+

Table 1. Continue.

94	Small rubber particle protein	gi|14423933	22/4.80	77	5/17	33	*Hevea brasiliensis*	cytoplasm	+
190	Peroxidase	gi|14029184	27/ 8.37	79	7/27	24	*Manihot esculenta*	response to stimulus	+
260	Phosphoenolpyruvate carboxylase	gi|22347639	109/6.12	75	16/58	17	*x Mokara* cv. *'Yellow'*	photosynthesis	+
2-41	Unnamed protein product	gi|9294584	38/9.00	77	11/70	41	*Arabidopsis thaliana*	unknown	↑
46	Os06g0136700	gi|115466258	92/7.08	77	11/39	13	*Oryza sativa (japonica cultivar-group)*	unknown	↑
47	Calcineurin B-like protein 9	gi|76577803	33/4.77	71	8/49	33	*Oryza sativa Japonica Group*	response to stimulus	↑
191	Phytochrome B	gi|57791644	130/5.66	76	17/67	17	*Arabidopsis thaliana*	metabolism	↑
234	hypothetical protein SORBIDRAFT_02g025080	gi|242044730	43/9.38	71	9/46	24	*Sorghum bicolor*	unknown	↑

a) MW and p*I* are theoretical. 0, 1 and 2 represent the ungrafted R.88-13, H.2/R.88-13 rootstock and R.88-13/ H.2 scion, respectively. +, −, ↑ and ↓ represent appearance, disappearance, up-regulation and down-regulation, respectively.

were intergrafted and used as a model to study the mechanism underlying rootstock-scion interactions in rubber tree. Proteomics is a powerful method to study biological processes. First using this method in the present study, a number of proteins related to rootstock-scion interactions of rubber tree were successfully identified.

Response to stimulus

In this study, some stress-related proteins which showed differential expression were identified, suggesting that rootstocks might lead to some extent of stress on their scions, and vice versa. Jensen et al. (2010, 2003) also found that many stress-related genes in apple scions were regulated by rootstocks. It is possible that there are interactions between rootstocks and scions of grafted rubber tree in stress tolerance.

Three protein spots were identified as heveins (Spots 0-67, 64 and 68), which only appeared in ungrafted R.88-13 and R.88-13/H.2 scion, but not in H.2/ R.88-13 rootstock. This result indicates that H.2 had significant effects on the expression of the proteins in R.88-13. Hevein is a chitin-binding protein which is known to be highly expressed in latex from rubber tree and involved in the co-agulation of rubber particles. Wounding, abscisic acid and ethylene could lead to the accumulation of hevein mRNA in leaves, stems, and latex but not in roots (Broekaert et al., 1990). In addition, hevein had also antifungal activity against several fungi *in vitro* (Parijs et al., 1991). The results of our study demonstrated that hevein could be also expressed in barks and may play an important role in rootstock-scion interaction of rubber tree.

The peroxidases, corresponding to spot 216 and 190, exist as isoenzymes and are heme-containing glycoproteins that utilize either hydrogen peroxide (H_2O_2) or oxygen (O_2) to oxidize various molecules (Yoshida et al., 2003)

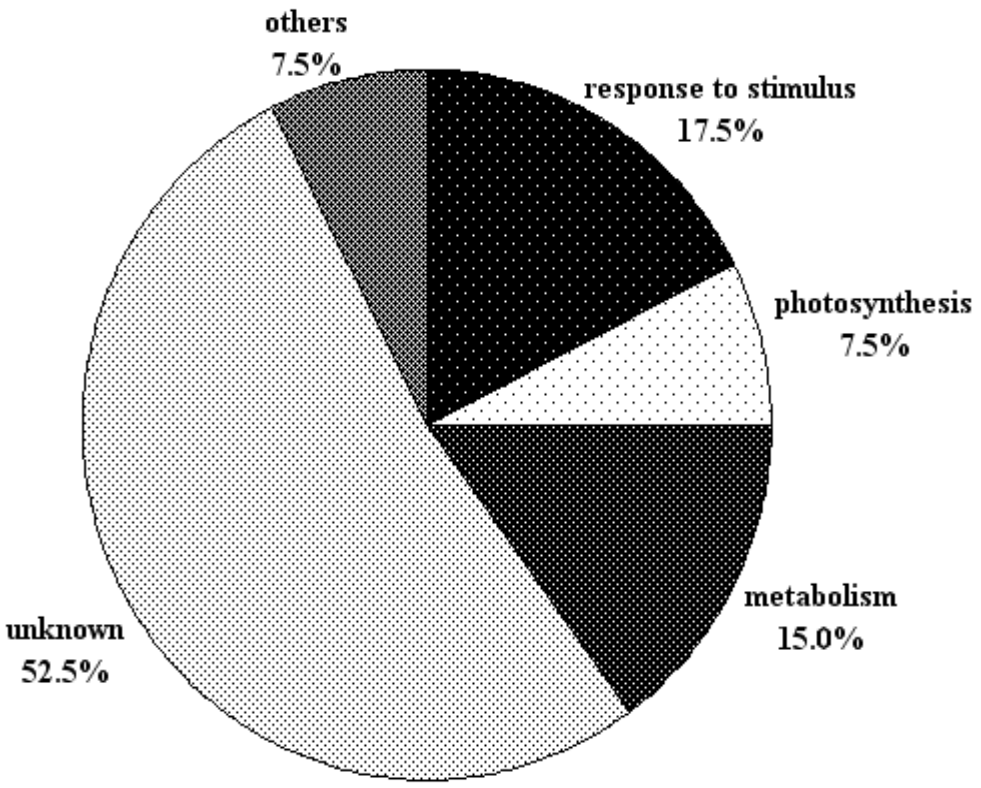

Figure 2. The functional category of the identified proteins.

and divided into three classes (class I, II and III) based on differences in primary structure in plants (Welinder, 1992). Studies have suggested that the plant peroxidases are involved in diverse physiological processes, such as scavenging of H_2O_2, auxin catabolism (Lagrimini et al., 1997), salt tolerance (Amaya et al., 1999) and defense against pathogen (Anjana et al., 2008). Huang et al. (2003) reported that the peroxidase isoenzyms from C-serums of rootstock and scion showed similar expression profiles in grafted *Hevea brasiliensis* different from the result in this study.

Spot 1-63 was matched with heat shock factor (Hsf). The plant Hsfs recognize the heat shock elements (HSEs) conserved in promoters of heat-inducible genes of all eukaryotes and function in the response of plants to various biotic and abiotic stresses. The over-expression of BhHsf1 could enhance thermotolerance and retard growth in both transgenic Arabidopsis and tobacco (Zhu et al., 2009). The HsfB2b was reported to be a negative regulator of defensin gene expression and pathogen resistance in Arabidopsis (Kumar et al., 2009). Additionally, Hsfs might act as the direct H_2O_2 sensors (Miller and Mittler, 2006).

Spot 47 had homology to calcineurin B-like protein 9 (CBL9). In plants, CBLs represent a family of calcium sensors that function in the calcium signaling. The CBL9 may function in abscisic acid (ABA) response (Pandey et al., 2004, 2008). However, the role of CBL9 in rootstock-scion interaction is unclear.

Metabolism

In this study, some metabolism-related proteins were successfully identified. These proteins may have key functions in rootstock-scion interactions.

Spot 37 was corresponding to theta class glutathione transferase GSTT1. Glutathione transferase was reported to play essential roles in plant primary and secondary metabolism, cell signaling and stress tolerance (Dixon et al., 2002). The differential expression of this enzyme after grafting may suggest its critical functions in rootstock-scion interactions. The taxadiene synthase (Spot 105) catalyzes the committed step in the taxol biosynthetic pathway (Walker and Croteau, 2001). The role of this protein in rootstock-scion interactions need to be further verified. Phytochrome B matched with spot 191 mediates photomorphogenesis and regulates plant growth and development (Rausenberger et al., 2010). The differential display of this protein may suggest that there are interactions between rootstocks and scions of grafted rubber tree in growth. The functions of the other metabolism-related proteins, such as putative mudrA protein (spot 1-62), mitochondrial F1-ATPase beta subunit (spot 231) and maturase K (spot 216) in rootstock-scion

interactions are still unknown.

Photosynthesis

Three photosynthesis-related proteins, spots 144, 145 and 260 matching with photosystem II protein 33kD, oxygen-evolving enhancer protein 1, and phosphor-enolpyruvate carboxylase, respectively were identified. The differential expression of these proteins implies the interaction between rootstock and scion in photosynthetic activity. It was also found that a large number of photosynthesis-related genes in apple scions were changed by the rootstocks (Jensen et al., 2003).

In addition, small rubber particle protein (SRPP) matching with spot 94 was reported to highly express in latex of rubber tree and play a positive role in rubber biosynthesis. This protein was not induced by ethylene and wounding (Oh et al., 1999). In this experiment, it was observed that SRPP expressed only in R.88-13/H.2 scion compared to H.2/ R.88-13 rootstock, suggesting that H.2 might increase the rubber yield of R.88-13/H.2 scion. This result further reflects the interactions between rootstocks and scions in yield (Ahmad, 1999; Cardinal et al., 2007).

Due to the difficulties in protein extraction of rubber tree barks, only about 300 protein spots are separated by 2-DE. Thus, improving the extraction method to separate more bark proteins is vital to comprehensive understanding the molecular mechanism underlying rootstock-scion interaction. At the same time, 10 protein spots were not successfully identified and many of the identified proteins were the hypothetical, predicted or unnamed ones probably due to the lack of rubber tree protein database. In following study, using tandem MS/ MS such as MALDI-TOF/TOF MS or ESI-MS-MS will be helpful to elevate the protein identification rate and the reliability.

In conclusion, rootstock-scion interaction is a complex physiological process, resulting in the expression of many differently functional proteins, such as stress, metabolism and photosynthesis. Although, some studies were made on rootstock-scion interaction, the molecular basis of rootstock-scion interaction has not been reported in rubber tree. The present study first validates the potential interaction between rootstock and scion of rubber tree in stress tolerance, growth and yield at the molecular level. In apple and tomato, it is also found that rootstocks alter gene expression patterns in their scions (Jensen et al, 2003, 2010; Zhang et al., 2008). Together with the results in our study, it can be suggested that rootstock-scion interaction is regulated by certain genes or proteins which play a crucial role in the interaction. Proteomics is a favorable technique which can be applied to identify proteins correlated with rootstock-scion interaction. According to the identified proteins, we postulate that some cell signaling may be directly involved in rootstock-scion interaction. For example, the identification of peroxidases and CBL9 implies that reactive oxygen species (ROS) and calcium signaling may be involved in rootstock-scion interaction. Further study of the identified proteins will facilitate the unraveling of the molecular mechanism underlying rootstock-scion interaction.

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